Oggetto	**POSTA CERTIFICATA: I: istanza accesso agli atti**
Mittente	"Per conto di: inmi[redacted]
Destinatario	<michele.rodaro[redacted]
Rispondi a	<inmi@[redacted]
Data	2021-06-28 13:00

SICUREZZA POSTALE
la posta elettronica certificata

- daticert.xml (~818 B)
- Elenco pubblicazioni inerenti isolati virali.docx (~16 KB)
- Protocollo 0007854 (1).pdf (~395 KB)
- postacert.eml (~564 KB)
- smime.p7s (~7 KB)

Messaggio di posta certificata

Il giorno 28/06/2021 alle ore 13:00:47 (+0200) il messaggio
"I: istanza accesso agli atti " è stato inviato da "inmi[redacted]
indirizzato a:
michele.rodaro[redacted]

Il messaggio originale è incluso in allegato.
Identificativo messaggio: opec2941.20210628130047.11089.879.2.68@pec.aruba.it

Oggetto	**I: istanza accesso agli atti**
Mittente	<inmi[redacted]
Destinatario	<michele.rodaro[redacted]
Data	2021-06-28 13:00

In relazione alla richiesta di accesso pervenuta in data 15 giugno 2021 si inoltra la mail di riscontro della dott.ssa Maria Rosaria Capobianchi, Direttore del Dipartimento di Epidemiologia clinica e diagnostica avanzata dell'INMI L. Spallanzani.
Cordiali saluti

Da: Capobianchi Maria Rosaria <maria.capobianchi[redacted]
Inviato: lunedì 21 giugno 2021 20:57
A: Direzione Sanitaria INMI Lazzaro Spallanzani <dirsan@inmi.it>
Oggetto: R: istanza accesso agli atti

In merito a quanto richiesto dall'Avvocato Rodaro, si rappresenta quanto segue.

Il richiedente usa il termine isolamento a sproposito.
In Virologia con il termine isolamento virale si intende **la messa in coltura di un campione biologico e la verifica della moltiplicazione del virus su un substrato di cellule vive permissive, coltivate in vitro.** L'isolamento si può ottenere anche in animali da esperimento, ma non è questo il caso.
Le cellule inoculate, in parallelo con un controllo non inoculato, vengono monitorate nel tempo per vedere se il virus cresce, la qual cosa è evidente come effetto citopatico, come presenza di particelle virali in microscopia elettronica, oppure, più comunemente, misurando nel tempo la quantità di genomi virali rilasciati dalle cellule in maniera progressivamente incrementale, come risultato della replicazione del virus. Non esistono altre accezioni del termine "Isolamento virale".

Il sequenziamento è tutt'altra cosa, e non va confuso con l'isolamento virale, in quanto è solo una lettura del genoma, e non misura la crescita del virus. Spesso si applica ai virus isolati per caratterizzarli, ma di per sé non equivale all'isolamento, che invece è un test di infettività.

All'INMI abbiamo isolato numerosi ceppi di SARS-CoV-2, messi a disposizione della comunità scientifica tramite piattaforme certificate (banche di virus); una di queste è EVAg, attraverso la quale abbiamo messo a disposizione 9 ceppi di SARS-CoV-2 isolati all'INMI e uno ottenuto da un altro laboratorio (https://www.european-virus-archive.com/evag-portal/field_product_type/virus-55/field_product_reference%253Afield_virus_host_type/human-virus-26366/field_product_reference%253Afield_country_of_collection/italy-25958/field_product_reference%253Afield_ictv_tax/severe-acute-respiratory-syndrome-related-coronavirus-22505.
I ceppi di SARS-CopV-2 isolati all'INMI sono stati utilizzati da altri laboratori a livello internazionale.

Non abbiamo atti da offrire per consultazione e non credo che il richiedente sia titolato a consultare registri di laboratorio; infatti chiede espressamente **l'elenco di documenti depositati.** A questo riguardo, nell'allegato sono riportati tutti i lavori che descrivono i risultati ottenuti all'INMI e le metodiche utilizzate che hanno comportato l'isolamento del virus, o l'uso di uno o più isolati virali per misurare fenomeni biologici quali l'effetto citopatogenetico (comprese alterazioni della morfologia cellulare evidenziata in microscopia elettronica in concomitanza con la presenza di particelle virali) , l'azione di sostanze biologiche e chimiche potenzialmente antivirali (compresi gli anticorpi naturali e monoclonali). Tali risultati sono stati pubblicati su riviste scientifiche a seguito di un processo di revisione da parte di esperti internazionali indipendenti, e sono tutti pubblicamente accessibili.
Il richiedente potrà agevolmente consultarli.

Maria Capobianchi

Maria R. Capobianchi

With regards to the access request received on the 15th June 2021, here we forward Ms Capobianchi Maria Rosaria's email, Director of the Department of Clinical Epidemiology and advanced diagnosis of INMI, L. Spallanzani.
Best regards.

From: Capobianchi Maria Rosaria <maria.capobianchi
Sent: Monday, 21st June 2021 20:57
To: Health Directorate INMI Lazzaro Spallanzani <dirsan@inmi.it>
Object: R: request for access the documentation

With reference to what requested from the attorney Mr Rodaro, here is the following.

The applicant uses the term "*isolation*" inappropriately.
According to virology, the term isolation shall mean the subsequently culturing of a virus' multiplication sample on a live permissive cell's substrate, cultured in vitro. The isolation can be also obtained with experimental animals, but this is not the case.
The inoculated cells, parallel to a non inoculated control, are monitored over time to see if the virus grows, which is evident as a cytopathic effect, like the presence of virus particles in E.M. or, more commonly, by measuring over time the quantity of viral genomes released by cells progressively incrementally as a result of the virus replication. There are no other meanings of the term "virus isolation".

The sequencing is something else, and it must not be confused with the virus isolation because it is only a reading of the genome, and it doesn't measure the virus growth. It is often applied to isolated viruses to characterize them, but it doesn't equal the isolation per se which is instead an infectivity test.

At INMI, we have isolated numerous strains of SARS-CoV-2, made available for the scientific community via certified platforms (virus banks); one of these is EVAg, through which we made available 9 strains of isolated 1SARS-CoV-2 from INMI, and one obtained from another laboratory (https://www.european-virus-archive.com/evag-portal/field_product_type/virus-55/field_product_reference%253Afield_virus_host_type/human-virus-26366/field_product_reference%253Afield_ictv_tax/severe-acute-respiratory-syndrome-related-coronavirus-22505.
The SARS-CoV-2 strains isolated at INMI have been used from other international laboratories.

We do not have any documentation to show for consultation, and I don not think the applicant is competent to look into the laboratory registers; in fact, he explicitly asks for the list of the registered documents. With this regard, in the annex, all the works showing the results obtained at INMI are listed with the methods used that led to the virus isolation or the use of one or more virus isolates to measure biological phenomena such as the cytopathogenic effect (including alterations of the cell morphology highlighted in electronic microscopy in conjunction with the presence of virus particles), the action of biological and chemical substances potentially antiviral (including natural and monoclonal antibodies). Such results have been published by scientific journals after a process of peer review from independent international experts, and they are all publicly accessible.
The applicant shall be able to consult them easily.

Maria Capobianchi

_____

Elenco (in ordine dal più recente al più vecchio) delle pubblicazioni con paternità INMI, in cui si riporta l'isolamento del virus da campioni clinici, l'uso di uno o più isolati virali per prove biologiche di danno cellulare, efficacia di potenziali antivirali, prove di sensibilità agli anticorpi neutralizzanti.

1: Amendola A, Garoffolo G, Songia P, Nardacci R, Ferrari S, Bernava G, Canzano P, Myasoedova V, Colavita F, Castilletti C, Sberna G, Capobianchi MR, Piacentini M, Agrifoglio M, Colombo GI, Poggio P, Pesce M. Human cardiosphere-derived stromal cells exposed to SARS-CoV-2 evolve into hyper-inflammatory/pro-fibrotic phenotype and produce infective viral particles depending on the levels of ACE2 receptor expression. Cardiovasc Res. 2021 May 25;117(6):1557-1566. doi: 10.1093/cvr/cvab082. PMID: 33705542; PMCID: PMC7989620.

2: Matusali G, Colavita F, Lapa D, Meschi S, Bordi L, Piselli P, Gagliardini R, Corpolongo A, Nicastri E, Antinori A, Ippolito G, Capobianchi MR, Castilletti C, Inmi Covid-Laboratory Team. SARS-CoV-2 Serum Neutralization Assay: A Traditional Tool for a Brand-New Virus. Viruses. 2021 Apr 10;13(4):655. doi: 10.3390/v13040655. PMID: 33920222; PMCID: PMC8069482.

3: Ciccosanti F, Di Rienzo M, Romagnoli A, Colavita F, Refolo G, Castilletti C, Agrati C, Brai A, Manetti F, Botta L, Capobianchi MR, Ippolito G, Piacentini M, Fimia GM. Proteomic analysis identifies the RNA helicase DDX3X as a host target against SARS-CoV-2 infection. Antiviral Res. 2021 Jun;190:105064. doi: 10.1016/j.antiviral.2021.105064. Epub 2021 Mar 26. PMID: 33781803; PMCID: PMC7997689.

4: Novelli G, Liu J, Biancolella M, Alonzi T, Novelli A, Patten JJ, Cocciadiferro D, Agolini E, Colona VL, Rizzacasa B, Giannini R, Bigio B, Goletti D, Capobianchi MR, Grelli S, Mann J, McKee TD, Cheng K, Amanat F, Krammer F, Guarracino A, Pepe G, Tomino C, Tandjaoui-Lambiotte Y, Uzunhan Y, Tubiana S, Ghosn J; COVID Human Genetic Effort; French COVID Cohort Study Group; CoV-Contact Cohort, Notarangelo LD, Su HC, Abel L, Cobat A, Elhanan G, Grzymski JJ,

Latini A, Sidhu SS, Jain S, Davey RA, Casanova JL, Wei W, Pandolfi PP. Inhibition of HECT E3 ligases as potential therapy for COVID-19. Cell Death Dis. 2021 Mar 24;12(4):310. doi: 10.1038/s41419-021-03513-1. PMID: 33762578; PMCID: PMC7987752.

5: Colavita F, Vairo F, Meschi S, Valli MB, Lalle E, Castilletti C, Fusco D, Spiga G, Bartoletti P, Ursino S, Sanguinetti M, Di Caro A, Vaia F, Ippolito G, Capobianchi MR. COVID-19 Rapid Antigen Test as Screening Strategy at Points of Entry: Experience in Lazio Region, Central Italy, August-October 2020. Biomolecules. 2021 Mar 13;11(3):425. doi: 10.3390/biom11030425. PMID: 33805832; PMCID: PMC7999510.

6: Nardacci R, Colavita F, Castilletti C, Lapa D, Matusali G, Meschi S, Del Nonno F, Colombo D, Capobianchi MR, Zumla A, Ippolito G, Piacentini M, Falasca L. Evidences for lipid involvement in SARS-CoV-2 cytopathogenesis. Cell Death Dis. 2021 Mar 12;12(3):263. doi: 10.1038/s41419-021-03527-9. PMID: 33712574; PMCID: PMC7952828.

7: Andreano E, Nicastri E, Paciello I, Pileri P, Manganaro N, Piccini G, Manenti A, Pantano E, Kabanova A, Troisi M, Vacca F, Cardamone D, De Santi C, Torres JL, Ozorowski G, Benincasa L, Jang H, Di Genova C, Depau L, Brunetti J, Agrati C, Capobianchi MR, Castilletti C, Emiliozzi A, Fabbiani M, Montagnani F, Bracci L, Sautto G, Ross TM, Montomoli E, Temperton N, Ward AB, Sala C, Ippolito G, Rappuoli R. Extremely potent human monoclonal antibodies from COVID-19 convalescent patients. Cell. 2021 Apr 1;184(7):1821-1835.e16. doi: 10.1016/j.cell.2021.02.035. Epub 2021 Feb 23. PMID: 33667349; PMCID: PMC7901298.

8: Rondinone V, Pace L, Fasanella A, Manzulli V, Parisi A, Capobianchi MR, Ostuni A, Chironna M, Caprioli E, Labonia M, Cipolletta D, Della Rovere I, Serrecchia L, Petruzzi F, Pennuzzi G, Galante D. VOC 202012/01 Variant Is Effectively Neutralized by Antibodies Produced by Patients Infected before Its Diffusion in Italy. Viruses. 2021 Feb 11;13(2):276. doi: 10.3390/v13020276.

PMID: 33670182; PMCID: PMC7916909.

9: Manzulli V, Scioscia G, Giganti G, Capobianchi MR, Lacedonia D, Pace L, Cipolletta D, Tondo P, De Nittis R, Rondinone V, Serrecchia L, Parisi A, Galante D, Lo Caputo S, Santantonio TA, Moschetta D, Dattoli V, Fasanella A, Foschino Barbaro MP. Real Time PCR and Culture-Based Virus Isolation Test in Clinically Recovered Patients: Is the Subject Still Infectious for SARS-CoV2? J Clin Med. 2021 Jan 15;10(2):309. doi: 10.3390/jcm10020309. PMID: 33467628; PMCID: PMC7829794.

10: Miersch S, Li Z, Saberianfar R, Ustav M, Case JB, Blazer L, Chen C, Ye W, Pavlenco A, Gorelik M, Perez JG, Subramania S, Singh S, Ploder L, Ganaie S, Chen RE, Leung DW, Pandolfi PP, Novelli G, Matusali G, Colavita F, Capobianchi MR, Jain S, Gupta JB, Amarasinghe GK, Diamond MS, Rini J, Sidhu SS. Tetravalent SARS-CoV-2 Neutralizing Antibodies Show Enhanced Potency and Resistance to Escape Mutations. bioRxiv [Preprint]. 2020 Dec 21:2020.10.31.362848. doi: 10.1101/2020.10.31.362848. PMID: 33398270; PMCID: PMC7781305.

11: Colavita F, Lapa D, Carletti F, Lalle E, Messina F, Rueca M, Matusali G, Meschi S, Bordi L, Marsella P, Nicastri E, Marchioni L, Mariano A, Scorzolini L, Ascoli Bartoli T, Di Caro A, Ippolito G, Capobianchi MR, Castilletti C; INMI COVID-19 Laboratory Team and INMI COVID-19 Study Group. Virological Characterization of the First 2 COVID-19 Patients Diagnosed in Italy: Phylogenetic Analysis, Virus Shedding Profile From Different Body Sites, and Antibody Response Kinetics. Open Forum Infect Dis. 2020 Sep 2;7(10):ofaa403. doi: 10.1093/ofid/ofaa403. PMID: 33527081; PMCID: PMC7499768.

12: Sauvat A, Ciccosanti F, Colavita F, Di Rienzo M, Castilletti C, Capobianchi MR, Kepp O, Zitvogel L, Fimia GM, Piacentini M, Kroemer G. On-target versus off-target effects of drugs inhibiting the replication of SARS-CoV-2. Cell Death Dis. 2020 Aug 19;11(8):656. doi: 10.1038/s41419-020-02842-x. PMID: 32814759; PMCID: PMC7434849.

13: Colavita F, Lapa D, Carletti F, Lalle E, Bordi L, Marsella P, Nicastri E, Bevilacqua N, Giancola ML, Corpolongo A, Ippolito G, Capobianchi MR, Castilletti C. SARS-CoV-2 Isolation From Ocular Secretions of a Patient With COVID-19 in Italy With Prolonged Viral RNA Detection. Ann Intern Med. 2020 Aug 4;173(3):242-243. doi: 10.7326/M20-1176. Epub 2020 Apr 17. PMID: 32302380; PMCID: PMC7175424.

14: Capobianchi MR, Rueca M, Messina F, Giombini E, Carletti F, Colavita F, Castilletti C, Lalle E, Bordi L, Vairo F, Nicastri E, Ippolito G, Gruber CEM, Bartolini B. Molecular characterization of SARS-CoV-2 from the first case of COVID-19 in Italy. Clin Microbiol Infect. 2020 Jul;26(7):954-956. doi: 10.1016/j.cmi.2020.03.025. Epub 2020 Mar 27. PMID: 32229288; PMCID: PMC7118617.

Dott. Fabio FRANCHI

Medico-Chirurgo
Specialista in:
- Igiene e Med. Preventiva
- Malattie Infettive
Società Scientifica per il Principio di Precauzione (SSPP), Italia
Già Dirigente Medico presso SC ospedaliero-universitaria
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TRIESTE

Oggetto: Replica alla risposta della professoressa Maria Rosaria Capobianchi (per l'INMI) alla richiesta di accesso agli atti (FOIA), inviata, in nome e per conto dell'Associazione **UHRTA TLT ODV** – United Human Rights Trieste Association, Territorio Libero di Trieste, Organizzazione di Volontariato – associazione per i diritti umani e del fanciullo di Trieste, dall'avvocato Michele Rodaro del Foro di Udine in data 15 giugno 2021. La risposta era inviata via PEC da INMI in data 28/06/2021.
Alla Direzione Sanitaria INMI Lazzaro Spallanzani
Prof./ssa Maria Rosaria Capobianchi

Gentilissima. Prof./ssa Capobianchi

La ringraziamo per la risposta alla richiesta di prove scientifiche a supporto della tesi dell'isolamento del virus SARS-CoV-2, e della bibliografia in allegato (i 14 l*avori "descrivono i risultati ottenuti dall'INMI e le metodiche utilizzate*" allo scopo).

Prima parte

Proponiamo una replica alla Sua risposta segnalandoLe che:

- 1) le spiegazioni da Lei gentilmente fornite non risolvono i dubbi da noi espressi circa l'insussistenza di elementi di prova richiesti,
- 2) l'esame attento del complesso delle informazioni reperibili nelle pubblicazioni scientifiche contenute nel Suo elenco fornisce la presenza di ulteriori elementi a favore della tesi del mancato isolamento.

Nel prosieguo di questa lettera proveremo a esporLe ordinatamente le ragioni che ci conducono alle due affermazioni precedenti.

Gentilmente ci rammenta preliminarmente che

> *"In Virologia con il termine isolamento virale si intende* ***la messa in coltura di un campione biologico e la verifica della moltiplicazione del virus su un substrato di cellule vive permissive, coltivate in vitro****".*

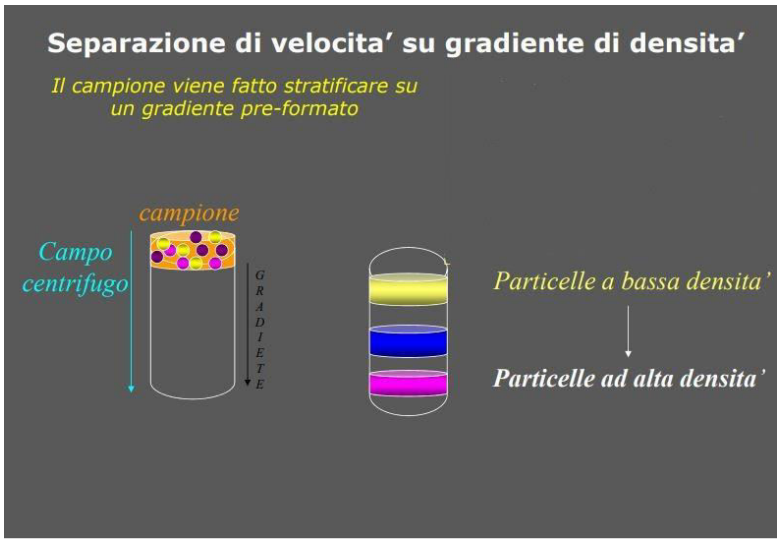
Ci ricorda anche che l'evidenza della presenza del virus è acquisita rilevando:

1. l'effetto citopatico in colture cellulari
2. la presenza di particelle virali evidenziabili con microscopia elettronica
3. come possibile alternativa, la misura "*nel tempo della quantità di genomi virali rilasciati dalle cellule*" in coltura.

A Suo parere, "*non esistono altre accezioni al termine "isolamento virale*".

Su questa definizione non siamo del tutto d'accordo per il motivo che non viene previsto l'isolamento fisico, che è la precondizione necessaria per le successive procedure di identificazione. Se questa tappa viene saltata, allora non vi è nessuna certezza su quanto viene poi determinato. Tale tappa risponde anche ad un requisito di logica elementare: prima di caratterizzare un qualcosa di sconosciuto, bisogna essere sicuri che si tratti proprio di ciò che si sta cercando, in modo da analizzare le varie componenti del solo agente cercato e non di altro. Come fare a separarlo?

In breve, è necessario: 1) filtrare il sopranatante della coltura presumibilmente infetta per levare i frammenti di maggiori dimensioni; 2) centrifugare in gradiente di densità al saccarosio che permette la separazione dei corpuscoli rimasti in vari strati (detti bande) in base alla loro densità; 3) procedere alla ripetizione dello stesso esame con le stesse identiche modalità da colture non infette; 4) esaminare con microscopia elettronica gli strati dove presumibilmente si sono depositati i virus cercati; 5) in caso siano visibili particelle similvirali "a tappeto" (nel primo esame, ma non nel controllo), analizzare le proteine e gli acidi nucleici contenuti in quello strato preciso; 6) effettuare prove di infezione di colture cellulari vergini con il materiale proveniente dallo stesso strato; 7) ripetizione di tutta la procedura. Per una descrizione più dettagliata si rimanda all'analisi di Papadopulos-Eleopuolos et al [1].



Se l'operazione completa va a buon fine allora si può parlare di isolamento di un virus (che per definizione deve essere appunto in grado di infettare e moltiplicarsi).

Ora - siamo d'accordo con Lei - la virologia moderna tende a evitare queste tappe essenziali: il probabile motivo è che darebbero risultati molto deludenti. Non utilizzandole, si ricorre ad altre metodiche per sostenere una dimostrazione che tale non è. Detto in altro modo, se si vuol sostenere che gli studi già pubblicati - anche quelli da Voi compiuti – soddisfino del tutto l'obiettivo dell'"isolamento virale", non ci dovrebbe essere alcun problema a ritrovare poi anche le particelle virali vere e proprie e non solo dei discutibili surrogati. La fotografia in microscopia elettronica (ME) di particelle similvirali in sezioni sottili di colture cellulari o tessuti non può essere sostitutiva della procedura menzionata per ragioni che saranno man mano più chiare.

I criteri a cui ci riferiamo esistono certamente, furono codificati all'Istituto Pasteur di Parigi e descritti anche da Françoise Sinoussi [2], Nobel per la Medicina nel 2008 assieme a Luc Montagnier. In sintesi, descrissero l'isolamento e la purificazione virale in gradiente di densità. Tali criteri sono stati disattesi in parte anche per l'isolamento dell'HTLV-III/LAV. Se ne parla qui perché le analogie sono fortissime e si tratta di un passaggio importante nella storia della virologia: segna anche il momento della svolta, dell'abbandono di certe regole. Le conferme del discostamento da esse arrivano da più fonti.

Una missiva di Mattew Gonda, il microscopista elettronico di Robert Gallo, resa nota al pubblico molti anni dopo, puntava il dito sulla **fallacia del riconoscimento tramite ME** da colture cellulari. Gonda aveva scartato la supposta identificazione virale – e spacciata come tale - perché quelle che aveva visto non erano altro che banali microvescicole, ritrovabili "in ogni agglomerato cellulare" [3]. Tra l'altro, Gonda le scarta anche per via delle dimensioni incompatibili, dimensioni che evidentemente contano e non solo per l'HIV. La lettera di Gonda fu spedita 3 giorni prima dell'invio per la pubblicazione delle prime foto del "virus" su Science [4]. In tale lavoro è espressamente specificato il metodo di isolamento fisico del virus come prima descritto [5]. Pur essendo ivi precisato che la maggior densità di

virus, visibile al ME (microscopio elettronico), si trovava nello strato corrispondente a 1,16 g/mL nel gradiente di densità, nessuna foto derivata da tale strato fu pubblicata allora. Anche Luc Montagnier menzionò l'isolamento fisico virale in gradiente di saccarosio nel 1983, nel suo primo lavoro sul LAV (HIV) [6], ed anche lui si guardò bene dal pubblicare le foto in ME dello strato sedimentato a 1,16g/mL. Quando, **14 anni dopo la "scoperta" dell'HTLV-III o LAV (HIV)** nel 1983-1984, due gruppi indipendenti di ricercatori effettuarono tali operazioni iniziali basilari (separazione e purificazione in gradiente di densità), si ritrovarono in mano (sotto il microscopio) … un pugno di mosche! Fuor di metafora, per oltre il 95% si trattava – secondo gli autori - di materiale cellulare eterogeneo (e solo rare erano le formazioni indicate come "virus" [7] [8], purtroppo neanche quelle poche ne possedevano le caratteristiche come evidenziato dal "Gruppo di Perth" [9]). Da tale materiale cellulare – a torto considerato fino ad allora "purificato virale" - erano stati ricavati tutti i test, il test anticorpale, quello antigenico e la PCR. Infatti nel 1997 i team di Bess e Gluschankof espressero preoccupazione che l'RNA e le proteine "usate per analisi biochimiche e sierologiche o come immunizzanti" originava dal materiale la cui purezza non era stata verificata.

Gli studiosi che più contribuirono ad analizzare e sviscerare questi aspetti fondamentali sono Eleni Papadopulos-Eleopulos, Valendar Turner et al. del "Gruppo di Perth" a cui va riconosciuto il merito principale [10], [11], [12] [13], [14], [15], [16] [17], [18]. Non risulta che siano mai stati contestati efficacemente.

Una conferma è arrivata dallo stesso Luc Montagnier, che in una famosa e mai smentita intervista dichiarò: "*Ripeto, noi non purificammo*" [19]. Era dunque ben consapevole che si poteva fare, ma non lo fece.

Quindi la metodica esiste ed è disponibile, perfettamente utilizzabile.

Lei scrive: "*Il sequenziamento è tutt'altra cosa, e non va confuso con l'isolamento virale, …*"

Ci teniamo anche a puntualizzare che nessuna confusione può esserci imputata al riguardo: una parte integrante dell'isolamento, una fase necessaria di esso, è la caratterizzazione degli acidi nucleici. Sono le prime fasi, quelle più importanti, che sono mancanti. Un *ipotetico* sequenziamento non può avvenire se non è stato dapprima separato il materiale genetico proveniente dallo strato di particelle similvirali.

Tornando alla Sua affermazione iniziale:

> *"In Virologia con il termine isolamento virale si intende la messa in coltura di un campione biologico e la verifica della moltiplicazione del virus su un substrato di cellule vive permissive, coltivate in vitro".*

Essa costituisce un problema anche per altri motivi. Infatti, se deve "*verificare la moltiplicazione del virus in coltura*", vuol dire che sa già cosa cercare. Ovvero lo conosce già, ovvero dà per scontato che il procedimento di riconoscimento sia già avvenuto correttamente nel passato e per tale motivo usa reattivi e

procedure già testati da altri ricercatori in precedenza. Purtroppo, dall'analisi della bibliografia sottostante i Vostri studi, consta rilevare che tali ricercatori che hanno operato prima di Voi non hanno fatto un buon lavoro. Nessuno ha neppure stabilito la relazione causale tra i risultati positivi ai test (equiparati, senza prove, a presenza di un nuovo virus) con la polmonite interstiziale bilaterale "COVID", avvalendosi dei postulati di Koch-Henle. Ciò è stato ammesso anche nei lavori iniziali di Zhu et al [20] e Zhou et al [21], spesso citati. Per inciso, e nel solo caso Lei non fosse d'accordo, Le sarà possibile segnalare le prime 3 pubblicazioni che, a Suo parere, lo abbiano stabilito con certezza.

Per quanto concerne l'isolamento, nessun passaggio di quelli che Lei ha elencato è specifico e può essere considerato come prova; si tratta di surrogati che non sono esclusivi, sia presi singolarmente che assieme.

L'**effetto citopatico** si può verificare per i più disparati motivi: evento dovuto a condizioni di coltura, azione di virus diversi e di batteri. Non permette di distinguere la causa. Persino Montagnier lo riconobbe [i], in relazione alla presunta citotossicità del virus HIV. E con qualche accorgimento indicò come evitarlo (con particolari antibiotici che Voi non avete usato in coltura, in Amendola et al., per esempio). Non è specifico neppure per il SARS-CoV-2.

La presenza di **particelle similvirali** in microscopia elettronica può essere fuorviante: sono presenti in sezioni sottili di molti tessuti, e pure di colture cellulari, specie quelle in sofferenza. Sicuramente le foto non possono essere spacciate per virus isolati (e neanche particelle isolate) in gradiente di densità. Il fatto che oggigiorno sia una prassi diffusa non significa necessariamente che vada bene. In questo contesto, bisogna fare attenzione a non usare il termine isolamento in modo improprio.

Che ci voglia anche l'isolamento fisico lo ha detto esplicitamente anche il virologo prof Ariberto Fassati [22] in una intervista rilasciata alla giornalista Gioia Locati de Il Giornale [23]: "*il virus non deve essere solo sequenziato, ma anche isolato fisicamente"*. Esistono altri metodi per farlo, oltre alla separazione in gradiente di densità? Non ci risulta.

La controprova è arrivata da due ricercatori [24] che hanno chiesto espressamente agli autori delle più importanti pubblicazioni scientifiche, nel cui titolo era menzionato il termine isolamento, se nelle fotografie al ME vi fossero i SARS-CoV-2 purificati. Le 4 risposte ottenute contenevano l'ammissione che ciò in effetti non era stato fatto.

Lei scrive: "***I genomi virali rilasciati dalle cellule in modo incrementale***". Secondo la teoria virale, le cellule non rilasciano solo genomi, ma soprattutto particelle virali (virus interi) in gran quantità. Come vengono in realtà rilevati e contati? **Con un test mai validato**, come dichiarato apertamente anche dal prof Giorgio Palù, Presidente dell'AIFA e della European Society for Virology, il 23 dicembre 2020, alla conferenza stampa voluta da Luca Zaia. Lo stesso è

[i] Djamel Tahi: intervista a Montagnier: "*Ed io controllai! Era un micoplasma, non un retrovirus."*

sostenuto da molti altri ricercatori. C'è un *consensus* su questo. **Il test non è neppure standardizzato** (come ammesso con gran ritardo a denti stretti anche dall'OMS, nel dicembre 2020 [25]: Secondo l'OMS, dunque, alti cicli di PCR, come ad esempio anche quelli da Voi usati nei lavori segnalati, sono in grado di positivizzare il "rumore di fondo", cioè qualsiasi cosa). Fin dai primi lavori pubblicati si era notata la grande erraticità delle risposte ai test Rt-PCR. Per esempio differenze nelle "cariche virali" non erano state trovate tra sintomatici ed asintomatici nel lavoro di Andrea Crisanti, pubblicato su Nature nel giugno del 2020 [26]. Ciò avrebbe dovuto costituire un problema interpretativo non di poco conto per i sostenitori della teoria virale (infatti la piena salute poteva andare tranquillamente a braccetto con "alte cariche" del virus mortale). Prendendo la questione da un altro punto di vista: **la positività del risultato del tampone-PCR per SARS CoV-2 non è necessario né sufficiente per la malattia** (intesa come polmonite interstiziale): esso può essere positivo in persone sane e negativo in una grossa quota di persone malate (e ricoverate per sospetta COVID, anche con polmonite interstiziale) [27]. Così è stato riscontrato a Wuhan e lo stesso è stato osservato anche in Italia [28]. Perciò altre ipotesi devono necessariamente essere considerate.

L'affidabilità dei test usati non è dunque una questione marginale, visto che è il perno della diagnosi, perciò converrà anche Lei che bisognerebbe avere un sufficiente grado di sicurezza su tutto quello che viene detto e fatto al riguardo. Ogni passaggio è importante.

Seconda parte

Brevi commenti riguardo le pubblicazioni presentate:

Tutti i lavori da lei gentilmente indicati nella sua bibliografia sono stati esaminati.

Non vengono analizzati qui in dettaglio, perché ciò porterebbe via troppo spazio, basti dire che nessuno di essi riporta l'isolamento fisico del virus come è stato a Voi richiesto. Inoltre in nessuno dei 14 lavori presenti nell'elenco da Lei allegato viene riportata la bibliografia di supporto all'affermazione ricorrente iniziale: "*nel gennaio 2020 un nuovo coronavirus fu identificato come la causa della polmonite*".

Era effettivamente un compito impossibile, visto che gli stessi CDC hanno ammesso con un documento ufficiale che non avevano disponibili i documenti richiesti dal FOIA [29]. Dalla risposta dei CDC: "*La definizione di "isolamento" fornita nella richiesta è al di fuori di ciò che è possibile in virologia, dato che i virus hanno bisogno delle cellule per replicarsi, e le cellule hanno bisogno di cibo liquido. Tuttavia, il virus SARS-Cov2 può essere isolato da un campione clinico umano mettendolo in coltura cellulare, che è la definizione di isolamento utilizzata in microbiologia…*"

Lei, professoressa Capobianchi, ha condiviso tale posizione, affermando: "*Non esistono altre accezioni del termine "isolamento virale"*. Eppure, come abbiamo

spiegato, il metodo di isolamento fisico esiste, è stato descritto in dettaglio, accettato dalla comunità dei Virologi, pur non essendo stato tentato con il "SARS-CoV-2" né da liquidi biologici prelevati da persone malate, e neppure con quello proveniente dalle colture infettate.

Dunque, verranno effettuate brevi osservazioni sui lavori da Lei allegati nella risposta (da bib 1 a bib 14), osservazioni che si integrano perfettamente con la nostra tesi.

1) **Amendola A** et al. (bib 1) [30]: lavoro pubblicato nel novembre 2020. Non vi è l'isolamento fisico del virus. Utilizza impostazioni già acquisite, dando per scontato che siano corrette, e su quelle è costruito il lavoro. L'effetto citopatico è aspecifico. Viene utilizzata la PCR fino a 40 cicli di amplificazione che allora sembrava potesse andare bene, ma ora è accettato anche dall'OMS [31] che non sia così. Così affermano anche altri esperti nel campo, ad esempio Bustin: "I programmi di test con *RT-qPCR per il SARS-CoV-2 sono completamente inadeguati, organizzati male e circondati da confusione e disinformazione".*[32]*.* Inoltre, in una precedente pubblicazione avevano affermato [33] *"noi dimostriamo che elementari errori di protocollo, inappropriata analisi dei dati e relazioni inadeguate continuano ad essere diffusi e concludiamo che la maggioranza dei dati pubblicati su RT-qPCR rappresentano più che altro artefatti (technical rumors)".*

2) **Matusali G** et al. (bib 2) [34]: nessun isolamento virale fisico effettuato. Gli autori sostengono che le prove di neutralizzazione con siero dimostrano come la protezione anticorpale persista per almeno 11 mesi, anche se vi è un calo del titolo. Quindi un buon risultato, apparentemente. Tuttavia quando viene fatto il confronto con il test per le IgG (anticorpi ritenuti specifici), si nota una quota considerevole di risultati negativi o molto bassi, tanto da indurre gli Autori a trovare altri *cutoff* di riferimento utilizzando unità arbitrarie (AU) al fine di aumentare prudenzialmente la sensibilità al 99% [ii] (a scapito della specificità, ridotta così al 29% [iii]). Specificità bassa significa accettare un altissimo numero di FALSI positivi. Quanti? Con una prevalenza ipotetica nella popolazione (poniamo 100.000 persone) del 2%, significa intercettare correttamente 1.980 positivi e non riconoscerne 20 (falsi negativi). Ma significa anche trovare solo 28.426 veri negativi. E gli altri? I rimanenti 69.594? Saranno scorrettamente identificati dal test. Come? Come positivi: 69.594 falsi positivi. In altre parole per ogni 36 positivi, 35 saranno falsi, utilizzando i dati degli Autori. Se le proiezioni fossero fatte su decine di milioni di italiani, i risultati sarebbero ancora più

[ii] Sensibilità: misura la capacità del test di individuare i veri positivi (VP/VP+FN)
Specificità: misura la capacità del test di individuare i veri negativi (VN/VN+FP)

[iii] Gli Autori scrivono: "However, with this cutoff, 14% of potential donors would have been lost (Table 1).
For this reason, we decided to adopt an IgG cutoff of 60 AU/mL (sensitivity 99%, 95%CI 94.8–100.0; specificity 29%, 95%CI 24.2–34.8), i.e., a more conservative value, to maximize the identification of adequate plasma donations, decreasing specificity in favor of sensitivity.

impressionanti. Il tutto con buona pace delle vittime innocenti ed inconsapevoli etichettate a torto come malate e costrette a quel ruolo. La domanda cruciale rimane senza risposta: come si fa a distinguere il risultato vero dal falso?

Nello studio di Chia et al [35], citato in Matusali, gli Autori riportano risultati problematici. Cioè che dei 164 pazienti seguiti, il 12% non aveva anticorpi neutralizzanti (cioè erano guariti senza "anticorpi protettivi") ed il 27% ne aveva, ma li perdeva però completamente nel giro di qualche mese. Gli Autori concludevano così: "*noi stabilimmo un algoritmo che considerava un ampio range di longevità degli anticorpi neutralizzanti, che variava da 40 giorni a molti decenni"*. Da "40 giorni"? Per essere più fedele ai dati da loro stessi proposti, l'algoritmo avrebbe dovuto considerare un range da zero in poi, o no?

Nello studio di Focosi et al, citato da Matusali et al., gli Autori scrivono: *"L'ampiezza della risposta anticorpale neutralizzante al SARS-CoV-2 è estremamente variabile, ed una significativa frazione di individui convalescenti hanno comparativamente livelli di anticorpi neutralizzanti plasmatici bassi o assenti.*" Citano anche la pubblicazione di Lei et al. così: "*i titoli di anticorpi neutralizzanti in individui asintomatici gradualmente spariva in due mesi.*" Gli autori non sembrano accorgersi che **anche ciò non è compatibile con la teoria virale**. È infatti accettato che la durata degli anticorpi, specie quelli attivamente formati, non possa essere di soli 2 mesi! Per esempio, gli anticorpi materni **(passivi)** sono ritrovabili nel neonato per 3-6 mesi.

Matusali et al. dimostrano insomma l'assoluta inadeguatezza dei test da loro presi in considerazione. In quale altra malattia virale gli anticorpi si comporterebbero in modo così "anomalo"? Bisognerebbe forse credere che le conoscenze basilari della immunologia non valgano più quando di mezzo c'è il SARS-CoV-2?

Che gli anticorpi si comportino in modo anomalo è stato confermato in dichiarazioni pubbliche anche dalla professoressa Capobianchi. In un'intervista pubblicata il 4 aprile 2020 [36], ha detto: "*con il test sugli anticorpi noi sappiamo solo che la persona si è infettata, ma non sappiamo quando, né se abbia risolto l'infezione*". Nel caso di morbillo o rosolia, guardando IgM e IgG si può dire se l'infezione è recente o no. Ma il SARS-CoV-2 sembra comportarsi diversamente. "*A differenza di altre infezioni in cui le IgM compaiono prima* – spiega Capobianchi – *per questo virus non si è osservata questa sequenza paradigmatica*". L'elenco delle stranezze sembra non finire mai.

Recentemente (il 24 agosto 2021) lo stesso prof Pregliasco ha confessato che le conoscenze al riguardo non sono molto migliorate nel tempo: "*Ad oggi - chiarisce Pregliasco - **non c'è una standardizzazione di test e non c'è un livello di anticorpi considerato protettivo.** Ci sono tecniche diverse, lo stesso campione con tecnologie diverse ha valori quantitativi numerici diversi. Non c'è un dato di riferimento. Si sta studiando, mancano ancora articoli scientifici. C'è bisogno - sottolinea il virologo - di approfondire meglio anche quali tipologie. Perché non c'è solo la quantità di anticorpi, ci sono gli anticorpi neutralizzanti, c'è*

l'attivazione dei linfociti B che è misurabile quindi bisogna consolidare alcune informazioni. Quando - osserva - se io dico che i miei anticorpi ora sono diventati niente dico una cosa spannometrica: ne avevo di più e ora sono calati moltissimo ma bisogna fare riferimento anche ad analisi eseguite nello stesso modo perché sennò hai degli choc".[37] Semplicemente: la confusione totale, dopo 20 mesi dall'inizio dell'avventura. Lo stesso Direttore Generale, Giovanni Rezza, del Ministero della Salute aveva sconsigliato di effettuare esami anticorpali ai fini del processo decisionale vaccinale [38], implicitamente **attribuendo loro assenza di valore protettivo**.

3) **Ciccosanti F** et al. (bib 3) [39]: non è soddisfatta la richiesta riguardo l'isolamento virale. La prima affermazione (*"… SARS-CoV-2, l'agente causale della COVID-19 …")* non è supportata da alcun riferimento bibliografico.

4) **Novelli G** et al. (bib 4) [40]: non è soddisfatta la richiesta riguardo l'isolamento virale.

La prima voce bibliografica citata è quella di Zhou P et al [19] i quali espressamente affermano che "*L'associazione tra 2019-nCoV e la malattia non è stata verificata da esperimenti su animali per soddisfare i postulati di Koch per stabilire una relazione causale tra il microrganismo e la malattia".* Non verificata negli animali e neppure nell'uomo evidentemente (i campioni esaminati provenivano da soli quattro pazienti - diconsi 4! -, e la PCR è stata usata con 40 cicli di replicazione. Ben lontani da una benché minima dimostrazione di relazione causale, dunque, che pretenderebbe ben altre prove.)

5) **Colavita F** et al. bib 5 [41]: non è soddisfatta la richiesta riguardo l'isolamento virale.

In questo lavoro gli Autori descrivono un test antigenico rapido da usare come screening paragonandolo ad altri. I risultati sono come minimo sconcertanti, in marcatissimo disaccordo tra loro [iv]. Nella figura 2 si può vedere quanti siano i casi di alta "carica virale", presumibilmente trovata con la NAAT (Nucleic Acid Amplification Test), associati ad assenza dell'antigene con il FIA (COI), e la marcata dispersione degli altri risultati:

[iv] Dei 603 risultati positivi al FIA (Fluorescence ImmunoAssay) COI (Cut Off Index), solo 34,3% era NAAT (nucleic acid amplification test) positivo e perciò il 65,7% da considerare falso positivo.

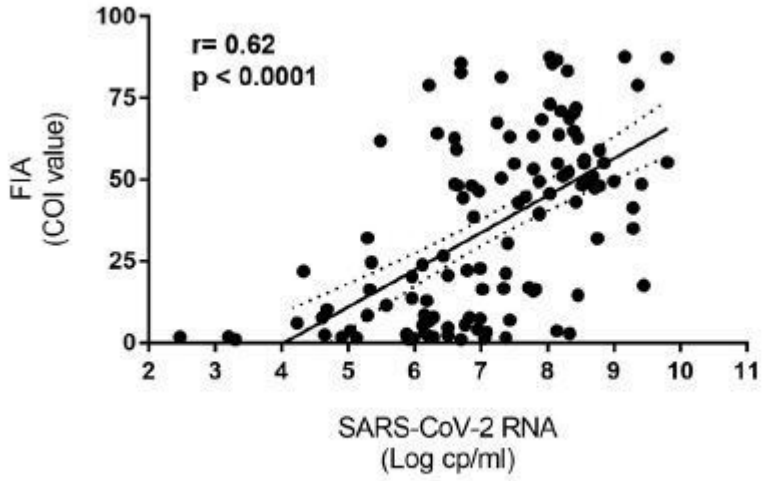


Figure 2. Correlation between SARS-CoV-2 RNA copies number and COI obtained on confirmed SARS-CoV-2 positive samples with available information for both parameters (n = 125, as in blue box

Uno dei test usati era stato testato in precedenza (Liotti et al. 2020 [42], citato da Colavita et al.) e dà una misura evidente della totale inaffidabilità dei risultati che si ottenevano. Per esempio in Liotti et al. è scritto che la percentuale di positività del FIA variava da 100% al 21%, in relazione al numero di cicli di amplificazione del NAAT (da <18 Ct a >35 Ct). Con i valori dichiarati per sensibilità e specificità[v] ed assumendo - come han fatto gli Autori - una prevalenza del 10% nella popolazione (poniamo 100.000 soggetti), si otterrebbero 6.150 risultati positivi di cui 1/3 FALSI (1.440). Tuttavia, se la prevalenza fosse dell'1%, come proposta dagli Autori (Colavita et al), i risultati sarebbero molto peggiori: 2.955 positivi di cui la stragrande maggioranza **FALSI (2.584, cioè 5,5 volte di più di quelli veri**). La sieroprevalenza, riscontrata in uno studio ad hoc effettuato in Italia, era del 2,5% nel luglio 2020 [43].

Come sono state trattate e conteggiate tali positività false? Come fossero vere infezioni, con relative quarantene, anche per i contatti. E blocchi di attività e lockdown a ripetizione con conseguenti danni alla salute fisica e psichica, oltre che all'economia.

Giustamente nel lavoro non si parla apertamente di sensibilità e specificità, ma di "*concordanza positiva e negativa*" dei risultati con il test NAAT (Rt-PCR), preso come riferimento. E ciò è corretto, poiché lo stesso NAAT, test di riferimento per l'OMS, non è mai stato validato. Quindi la reale sensibilità e specificità non possono essere determinate. La validazione dello stesso NAAT è stata effettuata internamente (cioè ripetendo il test) il che è da considerare una evidente distorsione da inclusione [44], molto poco scientifica. Da ciò deriva l'affermazione del Presidente dell'AIFA, prima menzionata.

6) **Nardac**ci R et al. (bib 6) [45]: non è soddisfatta la richiesta riguardo l'isolamento virale.

[v] Positive percent agreement (corrispondente a sensibilità): 47,1% Negative percent agreement (corrispondente a specificità): 98,4%. Con prevalenza "infezione" del 10% si avrebbero (su popolazione di 100.000 soggetti) 4.710 risultati veri positivi e 1440 falsi positivi. Con prevalenza dell'1%, si avrebbero 471 veri pos e 2,584 falsi positivi.

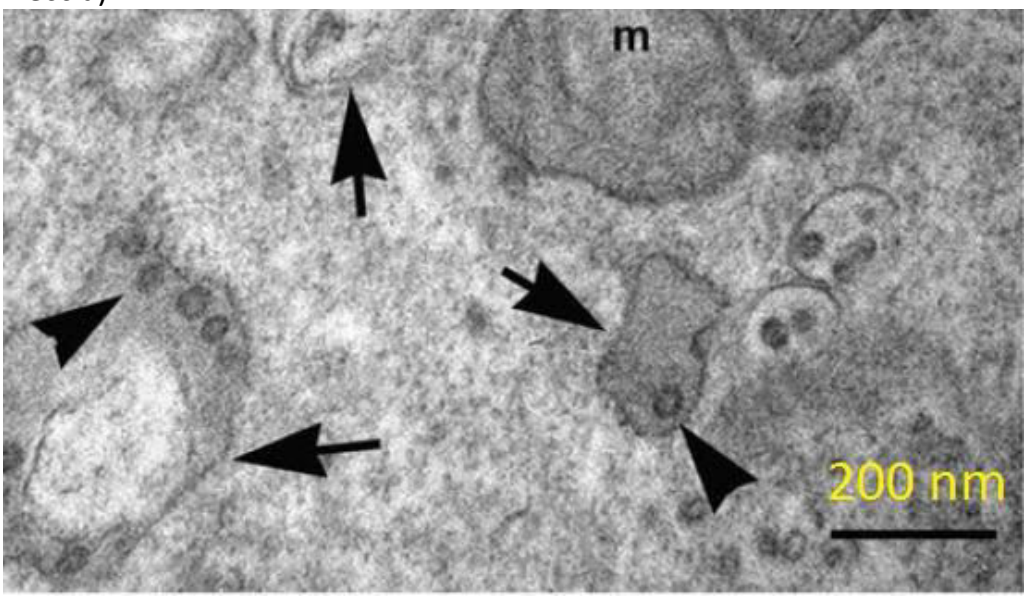
Gli autori scrivono: *"il diametro dei virus variava da 80 a 102 nm (misura media 93,61)"*.

È un punto molto importante dato che i virus, a differenza degli esosomi, devono avere una dimensione fissa, essendo costituiti per definizione da poche e precise componenti e non hanno una fase in cui sono cuccioli. Dovrebbero essere paragonati a gemelli identici (stesso corredo genetico con piccolissime variazioni).

La *International Committee on Taxonomy of Viruses* (ICTV) riporta che i Coronaviridae devono avere un diametro di 120-160 nm [46].

Quindi quelle immagini, che gli Autori hanno fotografato ed indicato con la punta delle frecce, NON possono essere coronavirus. Infatti i diametri dei "virus" [47] variano parecchio e la gran parte sono inferiori sia "al minimo sindacale" (ICTV), sia a quanto dagli Autori riportato nel testo (80-102 nm):

a) Nella fig 1A è di 75 nm,
b) Nell fig 1C varia tra 50 e 60 nm
c) Nella fig 1E: tra 60 e 70 nm
d) Nella fig 1F: circa 100 nm
e) Nella fig 2B: circa 50 nm
f) Nella fig2D: 75 nm
g) Nella fig 2F: da 50 a 70 nm
h) Nella fig 3 D: quelli indicati dalle frecce hanno 30-35 nm di diametro
i) Nella fig 3E: 35-40 nm ed uno 50 nm ("virus" indicati dalla testa della freccia)



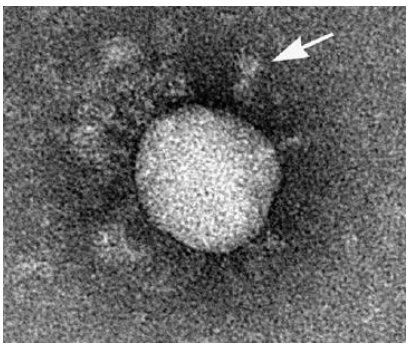
j) Nella fig 6 C: 100 nm

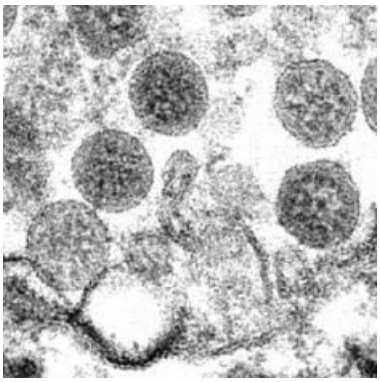
Val la pena ricordare che una particella con un raggio doppio rispetto ad un'altra ha un volume maggiore di 8 volte!

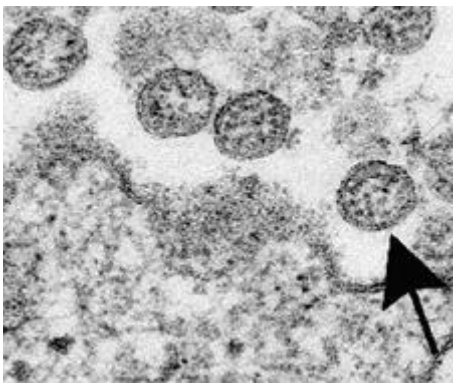
La numerosità di particelle con dimensioni ben inferiori a quelle minime attribuite ai Coronavirus e pure a quelle indicate dagli Autori, esclude si sia trattato di sviste od errori. Questo riscontro riporta in primo piano la discussione

sul come si faccia a stabilire cosa sia stato fotografato. Inoltre, in tale modo viene dimostrato che nessun isolamento virale è stato effettuato, visto che sicuramente molti di quelli, indicati dagli Autori con le punte di freccia, non possono proprio essere Coronavirus.

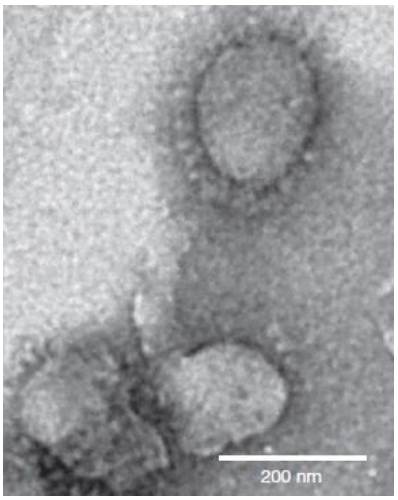
Per inciso, vi è notevole differenza anche nell'aspetto dei virioni, così come fotografati da Nardacci et al. e quelli fotografati da Goldsmith CS et al., dei CDC [48] e Hartcourt J et al, dei CDC [49], ad esempio. In questi ultimi **nessuna spike** – caratteristica da cui il coronavirus deriva il nome - è visibile). Eppure il virus – secondo quanto viene affermato – necessita delle estroflessioni per penetrare nelle cellule. Esse non sono opzioni, ma sono una parte integrante della struttura. Perciò non possono corrispondere alla definizione di coronavirus neanche quelli fotografati dai CDC.

 (Nardacci et al)

 (Goldsmith CS et al) no spikes

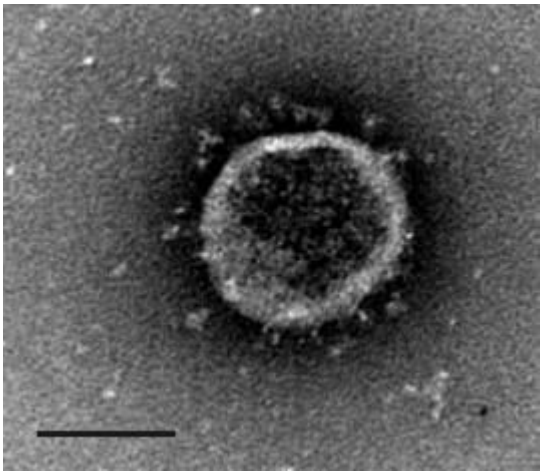
 (Hartcourt J et al) no spikes

In precedenza il Coronavirus, che era stato "isolato" (cioè fotografato da cellule in coltura), aveva il seguente aspetto definito peculiare, e dimensioni ben maggiori (Ge et al. [50]):



Il diametro del "virus" nell'immagine sopra (Ge et al) è circa 3,6 volte maggiore delle più piccole fotografate (50 nm) da Nardacci, Capobianchi et al. In termini di volume è **46,7 volte** più grande.

Niente in confronto al gigante di Bao et al. [51] i quali si sono vantati di aver soddisfatto i postulati di Koch, asseritamente infettando alcuni topi senza riuscire a farli ammalare. Il virus fotografato è questo:



La barra di riferimento è di 200 nm

Perciò il diametro della particella è 300 nm circa, quindi ha un volume del **21.500% maggiore rispetto ai piccoli "virus" italiani** (quelli di 50 nm di diametro, Nardacci et al.). Una differenza di non poco conto.

Un altro aspetto non trascurabile è questo: se i virioni hanno la stessa massa (hanno infatti le stesse componenti, non una di più, non una di meno), allora la densità delle particelle più grandi sarebbe molto minore delle più piccole, ovvero inversamente proporzionale al cubo del diametro. Ed anche questo non è digeribile, a meno che non si voglia accettare **il nuovo mistero gaudioso dei nuovi coronavirus: tanto diversi per forma, dimensioni, massa, densità e numero di varianti (oltre 3,3 milioni registrati sul GISAID per ora) eppur sempre tutti uguali**.

7) **Andreano E** et al. (bib 7) [52]: nessun isolamento virale fisico effettuato. Interessanti i risultati. È stato osservato che solo *"l'1,4% degli anticorpi*

neutralizzanti ritrovati (ndr: in pazienti guariti dalla COVID) *neutralizzavano il virus autentico*". È scritto proprio così. Un'altra stranezza da aggiungere all'elenco.

8) **Rondinone V** et al. (bib 8) [53]: nessun isolamento virale fisico effettuato. Il risultato trovato nello studio è interessante. Gli anticorpi di soggetti guariti dalla COVID avevano capacità di neutralizzare anche la "variante" inglese. Eppure la "variante" si è diffusa moltissimo tra gli "immunizzati" artificialmente. Una lezione da tener presente.

9) **Manzulli V** et al (bib 9) [54]: nessun isolamento virale fisico effettuato: gli Autori usano addirittura 45 cicli amplificazione con la PCR.

10) **Miersch S** et al. (bib 10) [55]: nessun isolamento virale fisico effettuato. Si parla degli anticorpi monoclonali come promettenti armi terapeutiche. Domanda: se gli anticorpi da vaccino, diretti contro antigeni selezionati, non servono contro le "varianti", perché dovrebbero servire i monoclonali? Per curiosità riportiamo le considerazioni al riguardo da parte del noto biologo molecolare, ex direttore dell'ECGEB a Trieste, prof Mauro Giacca [56]: *"La specificità di bersaglio che rende i monoclonali vincenti contro i tumori è anche il loro tallone di Achille nella lotta ai virus …*"

11) **Colavita F** et al. (bib 11) [57]: nessun isolamento virale fisico effettuato. La pubblicazione comincia con un errore: "*In January 2020, a novel coronavirus was identified as the cause of pneumonia cases, with the first cases reported in December 2019 in Wuhan City, Hubei Province of China [1, 2]*". Le voci bibliografiche[1, 2] non si riferiscono a procedure di isolamento, né a lavori dimostrativi della relazione causale [58]. A pagina 2 di Colavita et al c'è una sezione intitolata *"isolamento"*. In questo caso gli Autori si accontentano di osservare un effetto citopatico in colture cellulari inoculate con liquidi biologici da due persone presunte infette. **Niente microscopia elettronica, nessun controllo**. Non specificati gli antibiotici usati nelle colture. Per inciso, i due pazienti furono trattati con lopinavir/ritonavir (3 giorni) e remdesivir 13 giorni), che sono stati riconosciuti come farmaci inefficaci e non scevri di pesanti effetti avversi. Stranamente si tratta degli stessi pazienti descritti nella voce bibliografica 14 e lì l'esito dell'"isolamento" è dato come negativo per il paziente 2 (e non positivo come in bib 11).

12) **Sauvat A** et al. (bib 12) [59]: nessun isolamento virale fisico effettuato. Le prime affermazioni non sono supportate da alcuna pezza d'appoggio, in particolare: *"… the new SARS-CoV-2. This latter virus is causing a pandemic that started in 2019 and hence receives the name coronavirus disease-19 (COVID-19)"*.

13) **Colavita F** et al.[60]: nessun isolamento virale fisico effettuato. Nella prima frase si dà per scontato che la relazione causale tra COVID e SARS-CoV-2 fosse stata già determinata, ma non c'è alcun rimando bibliografico.

14) **Capobianchi MR** et al. (bib 14) pubblicato nel marzo 2020 [61]: il primo isolamento italiano. Nessun isolamento fisico effettuato. Di due casi (marito e

moglie, entrambi affetti da patologia respiratoria, entrambi positivi per la PCR), solo per uno vi fu positività della coltura e sequenziamento con NGS. La spiegazione fu che l'uomo aveva bassa carica virale (cicli di amplificazione 25). Tuttavia documenti dell'ISS considerano espressamente, per ottenere il sequenziamento delle varianti, campioni positivi per PCR con numero di cicli di amplificazione fino a 27 (considerato più che sufficiente in quanto a "carica"). La modalità di diagnosi si avvale da quanto proposto da Corman VM et al. su cui si impernia la modalità di diagnosi. Corman et al **hanno preparato i test senza avere il "virus" a disposizione**, si sono accontentati di fare il download via internet della sequenza trovata dai cinesi. Questo lavoro ebbe la review più veloce della storia della medicina, un vero Guinness dei primati: elaborato presentato il 21, accettato il 22 e pubblicato il 23 gennaio 2020. Tali e tanti sono i difetti del lavoro, che è stato richiesto il ritiro alla rivista (retraction) da parte di un gruppo di ricercatori [62], tra i quali anche Mike Yeadon, per molti anni direttore scientifico della Pfizer. Sebbene il provvedimento richiesto sia stato negato dalla rivista, la totale invalidazione del lavoro resta non confutata (viene allegata la review critica).

Conclusione

La lettera della professoressa Capobianchi e la bibliografia allegata paradossalmente forniscono ulteriori prove ed evidenziano come NON sia stato identificato correttamente un nuovo virus detto SARS-CoV-2. Non vi sono neppure stati tentativi di dimostrazione della relazione causale con la malattia (polmonite interstiziale).

Nessun accenno si riscontra nella lettera e nei lavori allegati della anomala definizione di caso, una specie di rete a strascico trainata da un test mai validato ed usato a tappeto. Tale definizione permette di effettuare la diagnosi anche in presenza di "brividi": se il risultato viene negativo, sono solo brividi, se viene positivo allora si tratta di malattia conclamata (COVID). Il che avrebbe dovuto suscitare qualche perplessità tra i clinici.

La mancanza di vero isolamento virale comporta la insostenibilità del significato attribuito ad ogni altro test (anticorpale, antigenico, molecolare) che a quello dovrebbe essere riferito. Ciò rende ragione delle mastodontiche incongruenze e discordanze riscontrate nella loro applicazione, nei correlati clinici e nella epidemiologia, e nella irrazionalità dei provvedimenti.

L'unico isolamento riuscito è stato quello di bambini, ragazzi, adolescenti, adulti, anziani e di un'intera società, deciso in base alle risultanze e conseguenze di una scienza in tal modo impostata.

Riteniamo che debba essere reso obbligatorio – invece che il vaccino – un ripensamento di tutta la materia, con la guida del metodo scientifico a cui Galileo Galilei diede la prima seria impostazione, pur ostacolato dalla Santa Inquisizione di allora.

Trieste, 8 settembre 2021

Ringraziamenti: sono in debito con il dott Luciano Macrì e con l'ing Roberto Serpieri per i loro utilissimi commenti e correzioni.

Allegati: Lettera prof Maria Rosaria Capobianchi + file pubblicazioni

Richiesta di ritiro ad Eurosurveillance della pubblicazione di Corman

Stefano Scoglio. La prova definitiva …18 giugno 2021

Bibliografia

[1] Papadopuols-Eleopulos E. et al. http://www.virusmyth.org/aids/hiv/epreplyek.htm

[2] Sinoussi F, Mendiola L, Chermann JC. Purification and partial differentiation of the particles of murine sarcoma virus (M. MSV) according to their sedimentation rates in sucrose density gradients. Spectra 1973; 4:237-243.

[3] Lettera di Gonda M a Popovic M 26 marzo 1984

MAR 27 REC'D

NATIONAL CANCER INSTITUTE

FREDERICK CANCER RESEARCH FACILITY

P.O. Box B, Frederick, Maryland 21701

March 26, 1984

Dr. Mika Papovic
Laboratory of Tumor Cell Biology
NIH
Building 37, Room 6B22
Bethesda, MD 20205

Dear Mika:

I am sending you 4 extra copies of results requested by Betsy Read. She said Dr. Gallo wanted these micrographs for publication because they contained HTLV particles. If this assumption is based on the cultures being antigen posstive, I would like to point out that the "particles" in micrograph 0905 are in debris of a degenerated cell. No other extracellular "virus-like particles" were observed free between cells anywhere in the pellet. The small extracellular vesicles in 0904 are at least 50% smaller than HTLV mature particles seen in type I, II, or III. Again, these vesicles can be found in any cell pellet. I do not believe any of the particles photographed are HTLV I, II, or III.

Best regards,

Matt

Matthew A. Gonda, Ph.D.
Head, Electron Microscopy Laboratory

MAG:jah

Enclosures

cc: Dr. Gallo
Betsy Read

PRI PROGRAM RESOURCES, INC. • Operations and Technical Support

Testo ingrandito (segue) →

Dear Mika:

I am sending you 4 extra copies of results requested by Betsy Read. She said Dr. Gallo wanted these micrographs for publication because they contained HTLV particles. If this assumption is based on the cultures being antigen positive, I would like to point out that the "particles" in micrograph 0905 are in debris of a degenerated cell. No other extracellular "virus-like particles" were observed free between cells anywhere in the pellet. The small extracellular vesicles in 0904 are at least 50% smaller than HTLV mature particles seen in type I, II, or III. Again, these vesicles can be found in any cell pellet. I do not believe any of the particles photographed are HTLV I, II, or III.

Best regards,

Matt

Matthew A. Gonda, Ph.D.

[4] Popovic M, Gallo R et al Science 1984;224:497-500

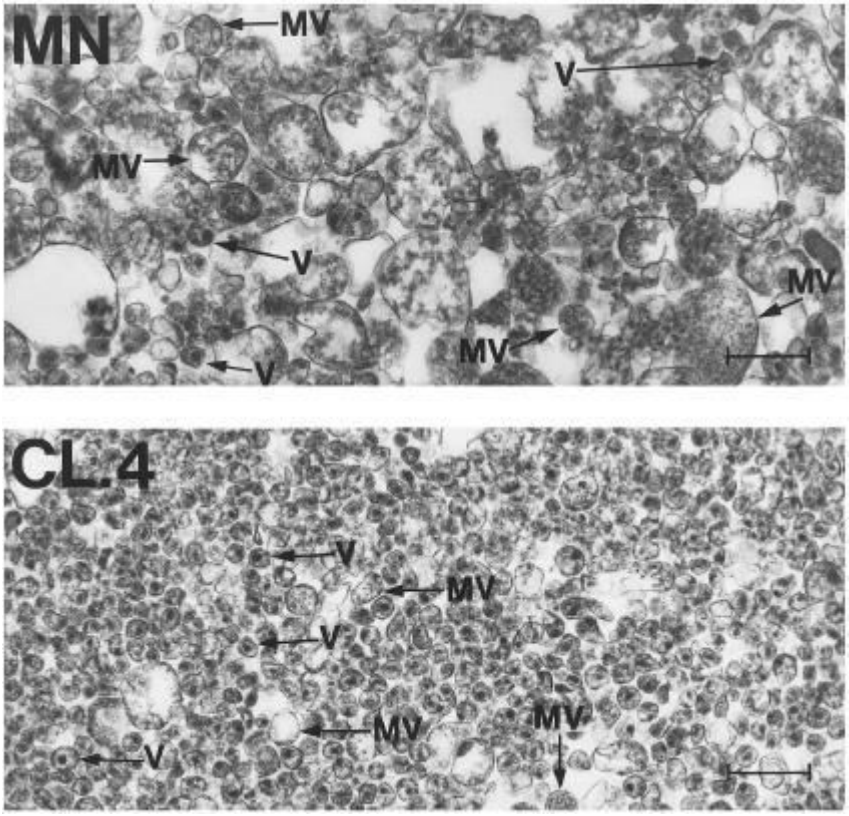
[5] Popovic M, Gallo R et al Science 1984;224:497-500

The yield of virus from H4/HTLV-III cells was assessed by purification of concentrated culture fluids through a sucrose density gradient and assays of particulate RT activity in each fraction collected from the gradient. As shown in Fig. 2b, the highest RT activity was found at a density of 1.16 g/ml, which is similar to other retroviruses. The highest RT activity was found in the fractions with the largest amount of virus, as determined by electron microscopy. The

[6] Barré-Sinoussi F et al. Science 1983;220:868

That this new isolate was a retrovirus was further indicated by its density in a sucrose gradient, which was 1.16, and by its labeling with [^{3}H]uridine (Fig. 1). Electron microscopy of the infected umbilical cord lymphocytes showed characteristic immature particles with dense crescent (C-type) budding at the plasma membrane (Fig. 2).

[7] "Virus isolato" in gradiente di densità. Le particelle virali sono indicate con "V". Bess GW et al. Virology 230, 134–144 (1997)



[8] Guschankof P et al. Virology 230, 125–133 (1997)

[9] Christine Maggiore Intervista a Eleni Papadopulos-Eleopulos http://www.virusmyth.com/aids/hiv/cjinterviewep.htm

[10] Papadopuolos-Eleopulos E. et al. http://www.theperthgroup.com/SCIPAPERS/EPEGalloProveRoleHIVEmergMedOCR1993.pdf Has Gallo proven the role of HIV in AIDS?
[11] Papadopuolos-Eleopulos E. et al. http://www.theperthgroup.com/SCIPAPERS/MHMONT.pdf A critique to Montagnier.
[12] Papadopulos-Eleopulos E, Turner VF, Papdimitriou JM. Is a Positive Western Blot Proof of HIV Infection? Bio/Technology 1993;11:696-707.
[13] Papadopuolos-Eleopulos E. et al. http://theperthgroup.com/LATEST/PGRevisitHIVExistence.pdf
[14] Papadopuolos-Eleopulos E. et al. http://www.theperthgroup.com/CONTINUUM/HaemophiliaConn.pdf haemophilia Connection
[15] Papadopuolos-Eleopulos E. et al. http://www.theperthgroup.com/CONTINUUM/PapadopolousReallyAchieved1996.pdf Isolation of HIV really achieved.
[16] Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM, Causer D. HIV antibodies: Further questions and a plea for clarification. Curr. Med. Res. Opin. 1997;13:627-634.
[17] http://www.virusmyth.org/aids/hiv/epreplyek.htm isolated facts about HIV a reply
[18] Papadopuolos-Eleopulos E. et al. http://www.theperthgroup.com/CONTINUUM/epeondjamel.html Commentary on Montagnier
[19] Djamel Tahi Intervista Luc Montagnier https://www.virusmyth.com/aids/hiv/dtinterviewlm.htm (allegato)
[20] Zhu N et al. N Engl J Med 2020;382:727-33. DOI: 10.1056/NEJMoa2001017
[21] Zhou P et al. Nature 2020; 579:270 12 March 2020
[22] Prof Ariberto Fassati. MD PhD della Division of Infection & Immunity, School of Medical Sciences, University College London
[23] Gioia Locati http://blog.ilgiornale.it/locati/2020/08/08/il-lockdown-sono-piu-efficaci-disciplina-e-igiene/
[24] Torsten Engelbrecht and Konstantin Demeter. COVID19 PCR Tests are Scientifically Meaningless . Bulgarian Pathology Association. https://bit.ly/34U60lA
[25] WHO https://www.who.int/news/item/14-12-2020-who-information-notice-for-ivd-users

WHO Information Notice for IVD Users 14 December 2020

Nucleic acid testing (NAT) technologies that use real-time polymerase chain reaction (RT-PCR) for detection of SARS-CoV-2

14 December 2020

"**In some circumstances, the distinction between background noise and actual presence of the target virus is difficult to ascertain.** Thus, the IFU will state how to interpret specimens at or near the limit for PCR positivity. In some cases, the **IFU will state that the cut-off should be manually adjusted to ensure that specimens with high Ct values are not incorrectly assigned SARS-CoV-2 detected due to background noise."**

[26] Lavezzo E, Neil M. Ferguson, Dorigatti I, Crisanti Andrea, Imperial College COVID-19 Response Team, et al. Nature https://doi.org/10.1038/s41586-020-2488-1.
[27] Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al. Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology. February 26, 2020; 1-23.
[28] F.Q. Coronavirus, il presidente del 118: "Casi Covid-like: polmonite interstiziale ma tampone negativo". Pregliasco: "Preoccupano" Il Fatto Quotidiano 17/05/2020.
[29] Stefano Scoglio. La prova definitiva che l'isolamento virale è una farsa 18 giugno 2021 (allegato).
[30] Amendola A, Capobianchi MR et al. Cardiovascular Research (2021) 117, 1557–1566

[31] OMS 14 dicembre 2020 vedi nota precedente.
[32] Bustin S and Nolan T. Int. J. Mol. Sci. **2020**, 21, 3004
[33] Bustin S and Nolan T Eur J Clin Invest 2017; 47 (10): 756–774
[34] Matusali, G. et al. SARS-CoV-2 Serum Neutralization Assay: A Traditional Tool for a Brand-New Virus. Viruses 2021, 13, 655.
[35] Chia WN et al. *Lancet Microbe* 2021; 2: e240–49
[36] Cristiana Pulcinelli.Maria Capobianchi: il test per gli anticorpi non è ancora affidabile. https://www.scienzainrete.it/argomenti/covid-19-intervista. Pubblicato il 04/04/2020
[37] ADNKRONOS. Intervista al prof Maurizio Pregliasco https://www.adnkronos.com/pregliasco-obbligo-vaccinale-per-over-40-o-green-pass-pesante_5y53TOLrsfvuwmY920fs1b 24 agosto 2021.
[38] Giovanni Rezza. **DIREZIONE GENERALE DELLA PREVENZIONE SANITARIA.** Ministero della Salute. **Vaccinazione dei soggetti che hanno avuto un'infezione da SARS-CoV-2. 3 marzo 2021**
[39] Ciccosanti F et al. Antiviral Research 190 (2021) 105064
[40] Novelli G, Capobianchi MR et al. Cell Death and Disease (2021) 12:310
[41] Colavita, F. Capobianchi MR et al. COVID-19 Rapid Antigen Test as Screening Strategy at Points of Entry: Experience in Lazio Region, Central Italy, August–October 2020. Biomolecules 2021, 11, 425.
[42] Liotti FM, Capobianchi MR et al. Clinical Microbiology and Infection 27 (2021) 487e488
[43] ISTAT Ministero Salute. PRIMI RISULTATI DELL'INDAGINE DI SIEROPREVALENZA SUL SARS-CoV-2. 3 agosto 2020
[44] Watson J et al. Interpreting a covid-19 test result. *BMJ* 2020;369:m1808 doi: 10.1136/bmj.m1808 (Published 12 May 2020)
[45] Nardacci R, Capobianchi MR et al. Cell Death and Disease (2021) 12:263
[46] ICTV Coronaviridae

[47] Misuratore su schermo: jruler.exe
[48] Goldsmith CS et al Lancet Vol 395 May 30, 2020.
[49] Hartcourt J et al. Emerging Infectious Diseases. www.cdc.gov/eid Vol. 26, No. 6, June 2020
[50] Ge et al. Nature 2013; 503:535
[51] Bao L et al. Nature 2020; 583:830.
[52] Andreano E et al. 2021, Cell 184, 1821–1835.
[53] Rondinone, V et al. Viruses 2021, 13,276.
[54] Manzulli, V., Capobianchi MR et al. Real Time PCR and Culture-Based Virus Isolation Test in Clinically Recovered Patients: Is the Subject Still Infectious for SARS-CoV2? J. Clin. Med. 2021, 10, 309.

[55] Miersch S et al. BioRxiv https://doi.org/10.1101/2020.10.31.362848; this version posted December 21, 2020.

[56] Il Piccolo, 17/05/2021

Dal virus al cancro Il grande successo dei monoclonali

MAURO GIACCA

Sono più di 20 gli anticorpi monoclonali già sperimentati o in fase di sviluppo contro Covid-19, alcuni dei quali hanno già ricevuto autorizzazione dalle agenzie regolatorie per l'uso di emergenza. Ma la specificità di bersaglio che rende i monoclonali vincenti contro i tumori è anche il loro tallone di Achille nella lotta ai virus, perché questi tendono a cambiare in continuazione, rendendo l'azione dell'anticorpo inefficace (è per questo motivo che il sistema immunitario reagisce alle infezioni virali producendo centinaia di anticorpi diversi contro tanti bersagli del virus anzichè una singola molecola). Tanto che già oggi, visto il successo del vaccino e l'insorgenza delle varianti, molte delle grandi biotec che producono monoclonali stanno interrompendo il loro investimento nel Covid per tornare alla ricerca di molecole sempre più efficaci, specialmente per la terapia dei tumori. —

[57] Colavita F et al. INMI COVID-19 Laboratory Team and INMI COVID-19 Study Group. Open Forum Infect Dis. 2020 Sep 2;7(10):ofaa403.

[58] Lake MA. What we know so far: COVID-19 current clinical knowledge and research. Clin Med (Lond) 2020; 20:124–7.
Velavan TP, Meyer CG. The COVID-19 epidemic. Trop Med Int Health 2020;25:278–80.

[59] Sauvat et al. Cell Death and Disease (2020) 11:656

[60] Colavita F et al. Annals of Internal Medicine. doi:10.7326/M20-1176.

[61] Capobianchi MR et al. Clinical Microbiology and Infection 26 (2020) 954e956

[62] Pieter Borger et al. 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 (documento allegato)

Dr Fabio FRANCHI

Medical Doctor
Specialised in:
- Hygiene and Preventive Medicine
- Infectious Diseases
Scientific Society for the Principle of Precaution (SSPP), Italy
Former Medical Manager c/o Struttura Complessa
universitaria-ospedaliera di Malattie Infettive, Trieste -Italy

Object: Reply to the answer of Professor Maria Rosaria Capobianchi (on behalf of INMI) to the request of access to documentation (FOIA), sent on 15th June 2021, on behalf of the Association **UHRTA TLT ODV** – United Human Rights Trieste Association, Free Territory of Trieste, Voluntary Organization – association for human rights and of the children of Trieste, from the attorney Mr. Michele Rodaro, Udine Jurisdiction. The answer was sent via PEC from INMI the 28/06/2021.

Simple summary

A FOIA was sent to the Spallanzani Institute (National Institute for Infectious Diseases – INMI - Rome), regarding the alleged isolation of SARS-CoV-2. Prof. Maria Rosaria Capobianchi, Director of the Clinical and Diagnostic Epidemiology Department of INMI, kindly replied to it, attaching 14 research articles to support her thesis.

She wrote to the applicant that the only means of achieving isolation in virology is to show: 1) a visible cytopathic effect on cell cultures, 2) presence of viral particles from cell cultures, 3) measure of the amount of viral genomes released by cells.

In this reply we object that all the above phenomena are non-specific and the only way to be sure is to physically isolate the virus. This is not only possible, but it is an accepted and standardized procedure in virology, also used for HIV isolation.

It consists in separating the presumed viral particles with ultra centrifugation in a density gradient of sucrose. The content of the corresponding band can be visualized with an electron microscope. If successful, the material in that band (pure virus) can be studied in its components, i.e. proteins, genetic code. Control tests are essential.

Despite more than 170,000 documents published on SARS-CoV-2 / COVID-19 in one year and a half, the above procedure has not been completed by anyone.

Among these documents, none showed a causal relationship between a positive PCR result and disease (interstitial pneumonia). The PCR test itself has never been validated or standardized, meaning no one knows what it identifies.

The publications in the list provided by prof Capobianchi fully confirm the absence of the required proof. Even more: they offer further proof that what have been recognized as SARS-CoV-2 particles cannot be coronavirus. They can't even be a single virus. In fact they are different in shape and size, often incompatible with the definition of coronavirus.

Furthermore, some of her papers show that the antigen test used, accepting all the parameters offered by the authors, gave rise to a huge number of false positive results (in a calculation, out of 36 positive results, 35 are false). Antibody tests are also very unreliable.

Lockdown and quarantine are founded on capriciousness of these tests.

To the Health Direction INMI Lazzaro Spallanzani
Prof. Maria Rosaria Capobianchi

Dear. Prof. Capobianchi

We thank you for the answer to the request of scientific proof in support of the thesis of the SARS-CoV-2 virus isolation, and for the bibliography attached

(the 14 works *"describe the results obtained at INMI and the methods used*" for the purpose).

First part

We propose a reply to your answer outlining that:

- 1) the explanations you gave do not solve the doubts we expressed about the non-existence of elements of proof requested,
- 2) the careful exam of all the information found in the scientific publications in your list gives the presence of further elements in favour of the thesis of the missing isolation.

In this letter we shall try to expose neatly the reasons that lead us to the two previous affirmations.

You kindly remind us that

> *"In Virology, the term virus isolation intends* ***the subsequently culture of a biological sample and the verification of the virus replication on a live permissive cell substrate, cultured in vitro****".*

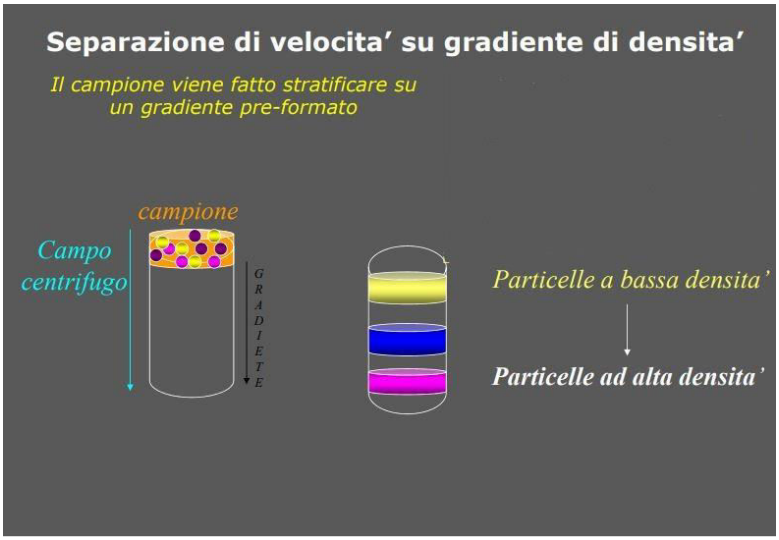
You also remind us that the evidence of the presence of the virus is acquired noting:

1. the cytopathic effect in cell cultures
2. the presence of viral particles demonstrable with electronic microscopy
3. as a possible alternative, the measurement "over time if the *quantity of viral genomes released by cells*" in culture.

According to you, "there are no other meanings *for the term "virus isolation*".

We do not completely agree on this definition for the fact that the physical isolation is not considered, despite it is the necessary precondition for the subsequent procedures of identification. If this step is missed, there is no certainty about what is then determined. This step also answers to the elementary logic requirement: before characterizing something unknown, you must be sure that what you found is just what you're looking for, so to analyse the various components of the only searched agent, and nothing else. How to separate it?

In short, it is necessary to: 1) filter the supernatant of the supposedly infected culture to remove the bigger fragments; 2) centrifugate in density gradient with sucrose allowing the separation of the left corpuscles in various layers (called bands) according to their density; 3) proceed and repeat the same exam in the same identical way from non infected cultures; 4) examine with electronic microscopy the layers where the searched viruses are probably stored; 5) in case virus-like particles are visible in a uniform layer (in the first exam, but not in the control), analyse the proteins and the nucleic acids in that specific layer; 6) carry out evidence of infection of virgin cell cultures with the material coming from the same layer; 7) repeat the whole procedure. For a more detailed description see the analysis of Papadopulos-Eleopuolos et al [1].



If the operation gives good results, then you can speak about isolation of a virus (that must be able to infect and to replicate itself by definition).

Now – we agree with you - modern virology tends to avoid those essential steps: the probable reason is that they would give very disappointing results. Without using them other methods are chosen to support a demonstration which is not true. In other words, if you want to support that the studies already published – even those you've made – satisfy the "virus isolation" goal completely, there shouldn't be any problem to then find again the viral particles themselves and not only some questionable surrogates. The imaging in electronic microscopy (EM) of virus-like particles in thin sections of cell or tissue cultures cannot replace the mentioned procedure for reasons that will be more and more clear.

The criteria to which we refer certainly exist; they were codified at the Pasteur Institute of Paris, and also described by Françoise Sinoussi [2], Nobel prize winner for Medecine in 2008 with Luc Montagnier. In short, they described the virus isolation and purification in density gradient. Such criteria have been dismissed partly also for the HTLV-III/LAV isolation. We're speaking about them here because the analogies are very heavy and it is an important stage in

the history of virology: it marks also the turning point of the abandonment of certain rules. The confirmations of that deviation come from several sources.

A missive by Mattew Gonda, Mr Robert Gallo's electronic microscopist, released to the public many years later, blamed the **fallacy of recognition via EM** from cell cultures. Gonda had discarded the supposed virus identification – sold as such – because what he had seen were no more than simple microvescicles, detectable "in each cell clusters" [3]. Inter alia, Gonda discards them for their incompatible dimensions as well that evidently count, and not only for HIV. Gonda's letter was sent 3 days before the dispatch for the publication of the first pictures of the "virus" on Science [4]. In this work, the virus physical isolation method is expressly specified as described above [5]. Even if it has been here specified that the greater density of virus, visible at the EM (electronic microscope), was in the correspondent layer at 1,16 g/mL in the density gradient, no picture derived from such layer was published then. Even Luc Montagnier mentioned the virus physical isolation in sucrose gradient in 1983, in his first work on LAV (HIV) [6], and even him never published the pictures in EM of the sedimented layer at 1,16g/mL. When, **14 years after the "discovery" of the HTLV-III or LAV (HIV) in 1983-1984**, two independent groups of researchers accomplished those basic initial operations (separation and purification in density gradient), they found (under the microscope) … nothing! The metaphor apart, for over 95% – according to the authors – it was heterogeneous cell material (and only rare formations indicated as "virus" [7] [8], alas not even those few got the characteristics as out lighted from the "Perth Group" [9]). From such cell material – until then wrongly regarded as "viral purified" - all tests had been derived, the antibody test, the antigenic one and the PCR. In fact, in 1997, the teams of Bess and Gluschankof expressed concern about RNA and proteins "used for biochemical and serology analysis or as immunizing" originated from the material with non verified purity.

The scientists who most contributed to analyse and eviscerate these fundamental aspects are Eleni Papadopulos-Eleopulos, Valendar Turner et al. from the "Perth Group" whose main merit must be recognised [10], [11], [12] [13], [14], [15], [16] [17], [18]. There is no record that they have ever been contested effectively.

A confirmation has arrived from Luc Montagnier himself, that in a famous and never invalidated interview declared: "I repeat*, we did not purify*" [19]. He was then well aware that it could be done, but he did not do it.

Hence, the method exists and is available, perfectly usable.

You write: "The *sequencing is something else, and it must not be confused with the virus isolation, …*"

We want to point out that no confusion can be charged to us in this regard: an integrating part of the isolation, a necessary phase of it, is the characterization of the nucleic acids. It's the first steps, the most important, that are missing. A hy*pothetical* sequencing cannot be if the genetic material has not been separated first from the virus-like particles layer.

Going back to your initial affirmation:

"In Virology, with the term virus isolation we mean the subsequently culturing of a biological sample and the verification of the virus replication on a permissive live cell substrate, cultured in vitro".

It constitutes a problem, even for other reasons. In fact, if you must "*verify the virus replication in culture*", it means that you already know what to look for. That is you already know it, i.e. you give for granted that the procedure of recognition has already correctly happened in the past, and for this reason you use reagents and procedures already tested from other researchers before. Unfortunately, from the underlying bibliography's analysis of your studies, we must observe that those researchers who operated before you have not done a good job. No one has even established the causal connection between the positive results to the tests (equated, without proof, to the presence of a new virus) and the interstitial bilateral pneumonia "COVID", using the Koch-Henle's postulate. This has also been admitted in the first works of Zhu et al [20] and Zhou et al [21], often mentioned. By the way, and only in case you would not agree, you will be able to report the first 3 publications that, according to you, have surely determined it.

For what concerns the isolation, none of the steps you listed is specific, and they cannot be considered as evidence; it's about surrogates that are not exclusive, either separately and together.

The cytopathic effect can be verified for number of reasons: event due to conditions of culture, action of different viruses and bacteria. It doesn't allow to distinguish the cause. Even Montagnier admitted it [i], in relation to the alleged cytotoxicity of HIV virus. And with some sort of expedient, he indicated how to avoid it (with specific antibiotics that you did not use in culture, in Amendola et al. for example). The cytopathic effect is not specific for SARS-CoV-2 neither.

The presence of **virus-like particles** in electronic microscopy can be misleading: they are present in thin sections of many tissues, as well as in cell cultures, especially those in distress. Surely, the pictures cannot be dealt like isolated

i Djamel Tahi: interview to Montagnier: "*And there I checked! It was a mycoplasma not a retrovirus."*

viruses (and isolated particles neither) in density gradient. The fact that nowadays it is a widespread practice does not necessarily mean that it's correct. In this context, one must be careful to not use the term isolation inappropriately.

Even virologist and professor Ariberto Fassati explicitly stated that the physical isolation is needed [22] during an interview with the journalist Gioia Locati of Il Giornale [23]: "the *virus must not only be sequenced, but also isolated physically"*. Are there other methods to do it, other then the separation in density gradient? We are not aware of them.

The check-test came from two researchers [24] who expressly asked the authors of the most important scientific publications where the title mentioned the term isolation, if in the images with the EM there were the purified SARS-CoV-2. The 4 answers they got provided the admission that they had not done it.

You write: "**The viral *genomes released by the cells in an incremental way"***. According to viral theory, the cells do not only release genomes, but above all viral particles (whole viruses) in big quantities. How are they actually detected and counted? **With a never validated test**, as openly declared even by prof Giorgio Palù, President of AIFA (corresponding to US FDA) and of the European Society for Virology, the 23rd December 2020, at the press-conference required by Luca Zaia (president of Veneto Region). The same is supported by many other researchers. There is a *consensus* on this. **The test is not even standardised** (as admitted by WHO very late and with gritted teeth, in December 2020 [25]: according WHO, then, high cycles of PCR, even like those you used in the reported works, are able to positivize the "background noise", i.e. anything). Since the first published works, the erratic results to PCR tests had been noticed. For example, differences in the "viral load" were not found between symptomatic and non symptomatic people in the study by Andrea Crisanti and Neil Ferguson, published on Nature in June 2020 [26]. This should have established a fairly considerable interpretation problem for the supporters of the viral theory (in fact good health could easily go hand in hand with "high loads" of the mortal virus). Looking at the issue from another point of view: **positivity of the result of the PCR test for SARS CoV-2 is not necessary nor sufficient for the disease** (read as interstitial pneumonia): it can be positive in healthy people and negative in a big share of sick people (and hospitalised for suspected COVID, even with interstitial pneumonia) [27]. That has been detected in Wuhan and the same has been observed in Italy, too [28]. So, other hypothesis must necessarily be considered.

The reliability of the used tests hence is not a marginal issue being the pivot for the diagnosis, so you must admit that one should have a sufficient degree of safety about all that is said on that regard. Every step is important.

Second part

Short comments concerning the presented publications:

All the works you kindly indicated in your list have been examined.

They are not hereby analysed in detail, because this would take too much space. Suffice it to say that none of them reports the physical isolation of the virus as we requested you. Furthermore, in any of the 14 works in the list you annexed, there is not one reference to support the initial recurring affirmation: "in January *2020, a new coronavirus was identified as the cause of the pneumonia*".

It was indeed an impossible task, as even CDCs have admitted in an official document that they did not have the requested documentation from FOIA [29]. From the CDC's answer: "The *definition of "isolation" provided in the request is out of what's possible in virology, because viruses need cells to replicate themselves, and cells need liquid food. Nevertheless, the virus SARS-Cov2 can be isolated from a clinical human sample putting it in a cell culture, which is the definition of isolation used in microbiology…*"

You, professor Capobianchi, have shared that position, stating: "*There are no other meanings for the term "virus isolation"*. Still, as we explained, the method of physical isolation does exists, it has been described in detail, accepted by the Virologists' community, even though it's not been tried with "SARS-CoV-2" not from biological liquids taken from sick people nor from that coming from infected cultures.

Hence, we will make short observations on the works you annexed in your answer (from ref 1 to ref 14 on your list), observations that integrate perfectly with our thesis.

1) **Amendola A** et al. (ref 1) [30]: study published in November 2020. there is no physical isolation of the virus. It uses already acquired settings, giving for granted that they are correct, and the study is built on those. The cytopathic effect is non-specific. PCR is used up to 40 cycles of amplification, which the seemed to work, but now even WHO accepted it is not [31] . The same is declared by other experts in the field, for instance Bustin: "The test programs with *RT-qPCR for SARS-CoV-2 are completely inadequate, badly organised and surrounded by confusion and disinformation".[32]. Moreover*, in a previous publication, they affirmed [33] *"we demonstrate that elementary errors of the protocol, inappropriate analysis of the data and inadequate relations continue to be spread and conclude that most of the published data on RT-qPCR represent mainly artefacts (technical rumours)".*

2) **Matusali G** et al. (ref 2) [34]: no physical isolation of the virus done. the authors support that the forecast antibody neutralisation for at least 11 months, even if there is a titre drop. Hence, a good result, apparently. However, when the comparison is done with the IgG test (antibodies considered specific), one can observe a considerable level of negative results or very

low, so much that the Authors had to find other reference *cutoff* using arbitrary units (AU) in order to prudentially raise the sensitivity to 99% [ii] (to the detriment of specificity, so lowered to 29% [iii]). Low specificity means accepting a very high number of FALSE positive. How many? With a hypothetical prevalence in the population (let's say 100.000 people) of the 2%, it means intercepting 1.980 correct positive and not recognising 20 (false negative). This also means finding only 28.426 true negative. And the others? The remaining 69.594? They will be incorrectly identified in the test. Like what? As positive: 69.594 false positive. In other words, **for each 36 positive, 35 will be false, using the data from the Authors**. If the projections were done on tens of millions of Italians, the results would be even more impressive. This, so much for the innocent and unconscious victims mistakenly labelled as sick and obliged to that role. The crucial question goes unanswered: how can one distinguish the true result from the false?

In the study by Chia et al [35], mentioned in Matusali, the Authors report problematic results. Namely, of the 164 followed patients, 12% did not have neutralising antibodies (I.e. they were healed without "protective antibodies") and 27% had them, but lost them completely within just a few months. The Authors concluded then: "we *set an algorithm that considered a wide range of longevity of the neutralising antibodies changing from 40 days to several decades"*. From "40 days"? In order to be more faithful to the data provided by the same authors, the algorithm should have considered a range from zero on, shouldn't it?

In the study by Focosi et al, mentioned by Matusali et al., the Authors write: *"The size of the neutralising antibody answer to SARS-CoV-2 is extremely variable, and a significant fraction of the convalescent individuals has comparatively low levels of neutralising plasma antibodies or absent.*" They cite the publications by Lei et al. as well: "the neutralising antibody titres *in individuals without symptoms gradually disappeared in two months.*" The authors do not seem to notice that **this is also incompatible with the viral theory**. It is indeed accepted that the antibodies' life, especially those actively formed, cannot last only 2 months! For example, maternal antibodies **(passive)** are detectable in the baby for 3-6 months.

Matusali et al. Then show the absolute inadequacy of the tests they have taken into account. What other infectious disease shows such "abnormal"

ii Sensitivity: it measures the capacity of the test to detect the true positive (VP/VP+FN)
Specificity: it measures the capacity of the test to detect the true negative (VN/VN+FP)

iii The Authors write: "*However, with this cutoff, 14% of potential donors would have been lost (Table 1). For this reason, we decided to adopt an IgG cutoff of 60 AU/mL (sensitivity 99%, 95%CI 94.8–100.0; specificity 29%, 95%CI 24.2–34.8), i.e., a more conservative value, to maximize the identification of adequate plasma donations, decreasing specificity in favor of sensitivity"*.

antibodies' behaviour? One should maybe believe that the basic knowledges of immunology do not count anymore with SARS-CoV-2?

Antibody abnormal behaviour has been confirmed in public declarations even by professor Capobianchi. In an interview published the 4th April 2020 [36], she said: "with the antibody *test, we only know that the person is infected, but don't know when nor if the infection is over*". In case of measles or rubella, *looking at* IgM and IgG one can say if the infection is recent or not. But SARS-CoV-2 seems to behave differently. "Unlike other *infections where IgM appear earlier* – Capobianchi explains – for this *virus this paradigmatic sequence has not been observed*". The list of oddities seems to be never-ending.

Recently (24th August 2021) prof Maurizio Pregliasco (an Italian TV "expert") himself has confessed that knowledge in this respect has not improved much over time: "*At present* – Pregliasco clarifies – ***there is no standardization of the tests and there isn't a level of antibodies considered protective.*** *There are different techniques, the same sample with different technologies has got different numerical quantitative values. There is no reference datum. We are studying, but scientific articles are still missing. It is necessary – underlines the virologist - to better expand which typologies. For there is not only the quantity of antibodies, there are the neutralising antibodies, there is the activation of the lymphocytes B which is measurable, so some information must be strengthened. When – he observes – if I say that my antibodies are now zero, I'm just guessing: I had more and now they have lowered a lot, but one has to refer also to the analysis done in the same way otherwise you have some shocks*".[37] Simply: we face a total confusion, after 20 months from the beginning of the adventure. The Director General himself, Giovanni Rezza, from the Health Ministry had discouraged antibody tests for the vaccine decision making [38], implicitly **not assigning them any protective value**.

3) **Ciccosanti F** et al. (ref 3) [39]: the request concerning the isolation of the virus is not satisfied. The first affirmation (*"… SARS-CoV-2, the causal agent of COVID-19 …")* is not supported by any bibliographical reference.

4) **Novelli G** et al. (ref 4) [40]: the request concerning the isolation of the virus is not satisfied.

The first mentioned bibliographic voice is Zhou P et al [19] who expressly affirm that "The *association between 2019-nCoV and the disease has not been verified with animal tests in order to satisfy the postulates of Koch to define a causal connection between the micro organism and the disease". Not* verified in animals nor in men evidently (the examined sample came from only four patients - just 4! -, and the PCR has been used with 40 cycles of replication. Far from any demonstration whatsoever of causal connection, that would pretend many more proofs.)

5) **Colavita F** et al. ref 5 [41]: the request concerning the isolation of the virus is not satisfied.

In this work the Authors describe an antigenic rapid test to use as screening comparing it to others. The results are at least disconcerting, in marked disagreement between them [iv]. In picture N.2 the cases of high "viral load", supposedly found with NAAT (Nucleic Acid Amplification Test), can be seen. They're associated to the antigenic absence with FIA (COI), and the marked dispersion of the other results:

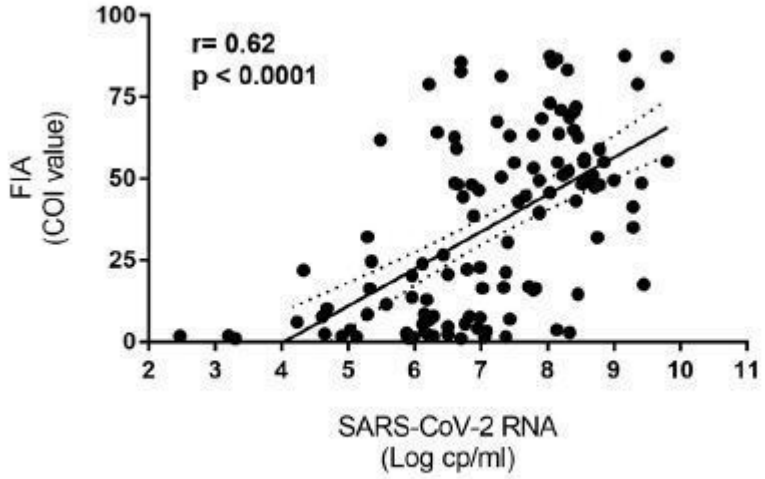


Figure 2. Correlation between SARS-CoV-2 RNA copies number and COI obtained on confirmed SARS-CoV-2 positive samples with available information for both parameters (n = 125, as in blue box

One of the used tests was previously tested (Liotti et al. 2020 [42], cited by Colavita et al.) and it gives an evident measurement of the total unaccountability of the results obtained. For example, in Liotti et al. It is written that the percentage of positivity of FIA changed from 100% to 21%, in relation to the number of cycles of amplification of NAAT (from <18 Ct to >35 Ct). With the declared values for sensitivity and specificity[v] , and assuming – as done by the Authors - a prevalence of 10% in the population (let's say 100.000 subjects), we would have 6.150 positive results, 1/3 of which FALSE (1.440). However, if the prevalence were 1%, as suggested by the Authors (Colavita et al), the results would be much worse: 2.955 positive, and the vast majority **FALSE (2.584, i.e. 5,5 times more than the real ones**). The seroprevalence, found in one study ad hoc carried out in Italy, was 2,5% in July 2020 [43].

How have those false positivity been treated and counted? As if they were real infections, including quarantine, also for the contacts. And activities blockage and lock-down over and over, with consequent damages to physical and psychological health, aside from economic aspects.

Rightfully, in the study, they do not openly speak about sensitivity and specificity, but of "positive and negative *concordance*" of the results with the

iv The 603 positive FIA results (Fluorescence ImmunoAssay) COI (Cut Off Index), only 34,3% was NAAT (nucleic acid amplification test) positive, so 65,7% to be considered as false positive.

v Positive percent agreement (correspondent to sensitivity): 47,1% Negative percent agreement (correspondent to specificity): 98,4%. With 10% of "infection" prevalence there would be (on a population of 100.000 subjects) 4.710 true positive results and 1440 false positive. With 1% prevalence, there would be 471 true positive and 2,584 false positive.

NAAT test (Rt-PCR), taken as reference. And that is correct, because the same NAAT, reference test for the WHO, **has never been validated**. So, real sensitivity and specificity cannot be determined. The validation of the same NAAT has been carried out internally (i.e. repeating the test) which must be considered an evident inclusion bias [44], very unscientific. This is where the affirmation of the President of AIFA, aforementioned, is derived from.

6) **Nardac**ci R et al. (ref 6) [45]: the request concerning the isolation of the virus is not satisfied.

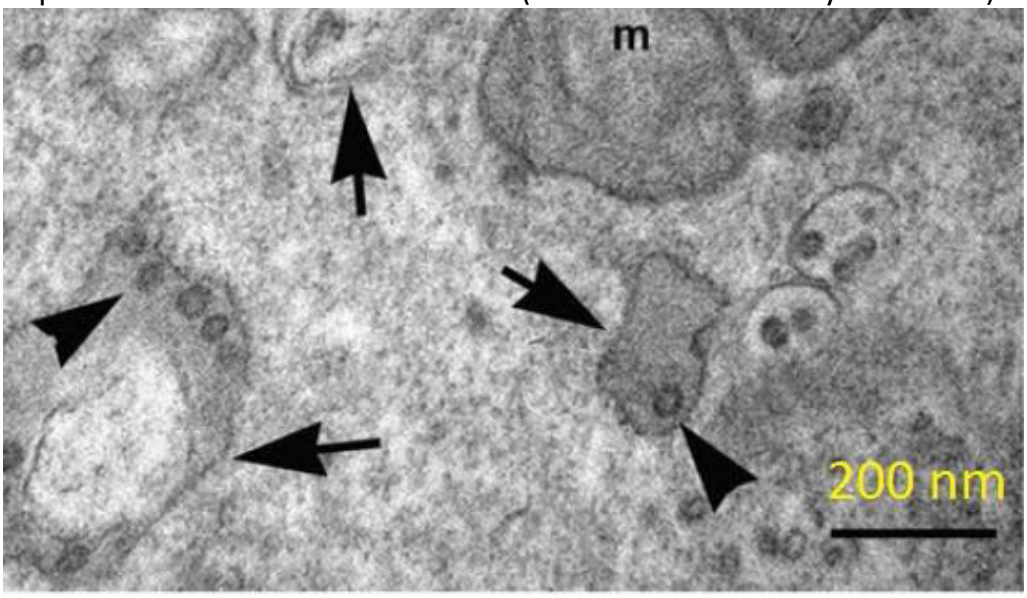
The authors write: *"the diameter of the viruses changed from 80 to 102 nm (average size 93,61)"*.

it is a very important point as the viruses, unlike exozomes, must have a fixed dimension being constituted of few and precise components per definition, and they don't have a phase where they are babies. They should be compared as identical twins (same genetic endowment with very little variations).

The *International Committee on Taxonomy of Viruses* (ICTV) reports that Coronaviridae must have a diameter of 120-160 nm [46].

Hence those images that the Authors have taken and indicated with the arrows, CANNOT be coronavirus. In fact, the diameters of "virus" [47] vary a lot and the major part are inferior to both "the minimum wage" (ICTV) and to what the Authors reported in the text (80-102 nm):

a) In pict. 1A, it is 75 nm,
b) In pict 1C, it varies between 50 and 60 nm
c) In pict. 1E: between 60 and 70 nm
d) In pict, 1F: around 100 nm
e) In pict. 2B: around 50 nm
f) In pict. 2D: 75 nm
g) In pict. 2F: from 50 to 70 nm
h) In pict. 3 D: the ones indicated by the arrows have 30-35 nm diameter
i) In pict. 3E: 35-40 nm and one 50 nm ("viruses" indicated by the arrow)

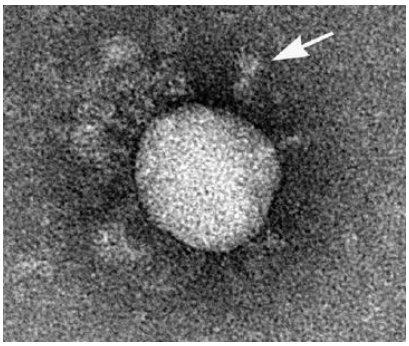


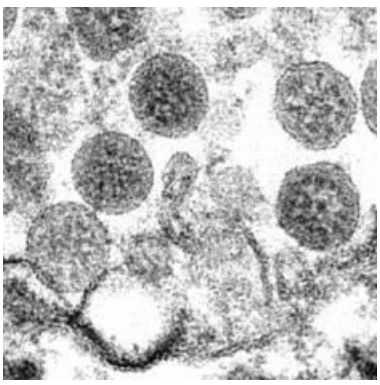
j) In pict. 6 C: 100 nm

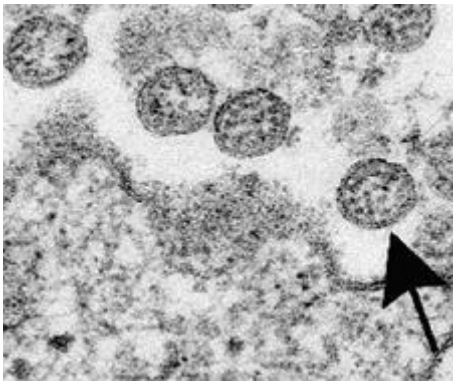
It is important to recall that a particle with a radius twice as large as another one has a volume 8 times bigger!

The abundance of particles with dimensions far below to the minimum attributed to Coronaviruses as well as to those indicated by the Authors, excludes that it was an oversight or an error. This finding foregrounds the discussion on how to establish what was photographed. Furthermore, in this way, it is shown that no virus isolation has been carried out, because surely many of those, indicated by the Authors with the arrow, cannot be Coronaviruses.

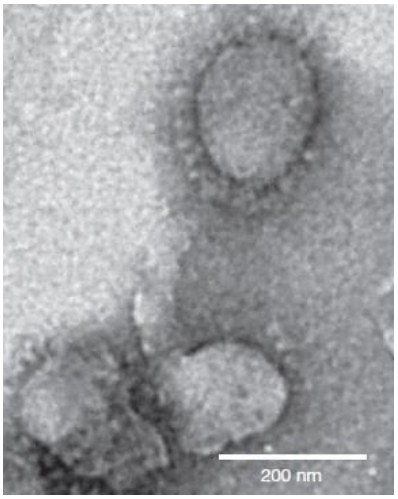
Incidentally, there is also a huge difference in the virions' aspect, as they were photographed by Nardacci et al. and the ones photographed by Goldsmith CS et al., by CDC [48] and Hartcourt J et al, by CDC [49], for example. In these last ones **no spike – characteristic from which coronavirus derives its name - is visible**). Yet the virus – as mentioned – needs outgrowths to penetrate the cells. These are not options, but an integral part of the structure. Therefore, even the ones photographed by CDC cannot correspond to the definition of coronaviruses.


(Nardacci et al)


(Goldsmith CS et al) no spikes

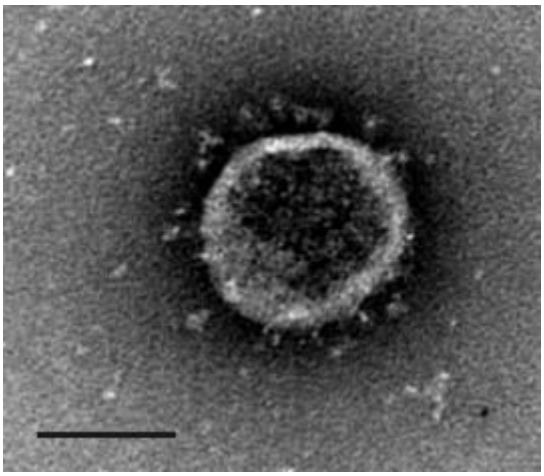

(Hartcourt J et al) no spikes

Previously, Coronavirus, that had been "isolated" (i.e. photographed from cultured cells), had the following look, defined peculiar, and dimensions much bigger (Ge et al. [50]):



The diameter of the "virus" in the above imagine (Ge et al) is around 3,6 times bigger then the smallest photographed (50 nm) by Nardacci, Capobianchi et al. In terms of volume, it is **46,7 times** bigger.

Nothing if compared to the giant of Bao et al. [51] who have bragged about having satisfied the postulates of Koch, allegedly infecting some mice without making them sick. The photographed virus is this:



The reference bar is 200 nm

Hece, the diameter of the particle is 300 nm ca, so to say it has a volume **21.500% bigger in relation to the small Italian "viruses"i** (the ones with a 50 nm diameter, Nardacci et al.). A fairly considerable difference.

Another not inconsiderable aspect is this: if the virions have got the same mass (they have in fact the same components, not one more, nor one less), then, the density of the bigger particles would be much lower than the smallest, i.e. inversely proportional to the diameter cube. And this is also not digestible, unless one would want to accept **the new joyful mistery of the new coronaviruses: so much different in shape, dimensions, mass, density and**

number of variants (more than 3,3 millions registered on GISAID at the moment) but still all the same.

7) **Andreano E** et al. (ref 7) [52]: no physical isolation of the virus carried out. Interesting results. T has been observed that only "*1,4% of the neutralising antibodies found* (ndr: in patients healed from COVID) *neutralised the real virus*". It has been really written. Another oddity to be added to the directory.

8) **Rondinone V** et al. (ref 8) [53]: no physical isolation of the virus carried out. The result of the study is interesting. The antibodies of healed subjects from COVID were able to neutralise even the "English variant". Yet, the "variant" spread widely among the artificially "immunised". One lesson to take into consideration.

9) **Manzulli V** et al (ref 9) [54]: no physical isolation of the virus carried out: the Authors even use 45 cycles amplification with PCR.

10) **Miersch S** et al. (ref 10) [55]: no physical isolation of the vrus carried out. It deals with monoclonal antibodies as promising therapeutic weapons. Question: if the vaccine antibodies, directed against selected antigens, do not work against the "variants", why should the monoclonals work? Out of curiosity, we report the considerations on this matter from the well known molecular biologist, former director of ECGEB in Trieste, prof Mauro Giacca [56]: *"The specificity of the target making monoclonals successful against cancers is also their weak point against viruses …*"

11) **Colavita F** et al. (ref 11) [57]: no physical isolation of the vrus carried out. The publication starts with an error: "*In January 2020, a novel coronavirus was identified as the cause of pneumonia cases, with the first cases reported in December 2019 in Wuhan City, Hubei Province of China [1, 2]*". The bibliographic references [1, 2] don not refer to procedures of isolation nor to demonstrative works of the causal connection [58]. At page 2, in Colavita et al., there is a section called *"isolation"*. In this case the Authors are satisfied with the observation of a cytopathic effect in cell cultures inoculated with biologcal liquids from two people supposedly infected. **No electronic microscopy, no control**. The antibodies used in the cultures are not specified. By the way, the two patients were treated with lopinavir/ritonavir (3 days) and remdesivir 13 days), which have been recognised as ineffective drugs, and not without heavy adverse reactions. Strangely, it's the same patients described in bibliography 14 and there, the result od the "isolation" is negative for patient 2 (and not positive as in ref 11).

12) **Sauvat A** et al. (ref 12) [59]: no physical isolation of the virus carried out. The first affirmations are not supported by any documentation, in particular: *"… the new SARS-CoV-2. This latter virus is causing a pandemic that started in 2019 and hence receives the name coronavirus disease-19 (COVID-19)"*.

13) **Colavita F** et al.[60]: no physical isolation of the virus carried out. In the first sentence, the causal connection between COVID and SARS-CoV-2 is taken for granted, but there is no bibliographic reference.

14) **Capobianchi MR** et al. (ref 14) published in March 2020 [61]: the first Italian isolation. No physical isolation of the virus carried out. Two cases (husband and wife, both with respiratory disease, both positive to the PCR), only one had positivity of the culture and sequencing with NGS. The explanation was that the man had a low viral load (cycles of amplification 25). However, documents of the ISS (ndt, Health National Institute) expressly consider positive samples for PCR in order to obtain the sequencing of the variants, with numb er of cycles of amplification up to 27 (considered more than sufficient because with "load"). The diagnostic mode uses what proposed by Corman VM et al. pivoting the diagnosis method. Corman et al **have prepared the tests without having the "virus" available**, they were satisfied with the download via internet of the sequence found by the Chinese. This study had the fastest review ever in medicine history, a real Guinness record: elaborated, presented the 21st, accepted the 22nd and published the 23rd January 2020. The defects are so numerous in the study that it has been asked to the journal (retraction) to withdraw it from a group of researchers [62], among them **Mike Yeadon** as well, scientific director of Pfizer for many years. Although the measures requested have been denied from the journal, the total invalidation of the study stands unrefuted (see the critic review annexed).

Conclusion

Prof Capobianchi's letter and the annexed bibliography, paradoxically provide further evidence, and highlight how no new SARS-CoV-2 virus has EVER been identified correctly. There have not even been attempts to demonstrate the causal connection with the disease (interstitial pneumonia).

No sign is noticed in the letter and the annexed studies of the abnormal definition of the case, some kind of trawl towed by a never validated test. This definition allows to carry out diagnosis even in presence of "chills": if the result is negative, it's only chills, if it's positive they must be considered as expression of the disease (COVID). This should have caused some perplexity among clinicians.

The lack of real virus isolation leads to the unsustainability of the attributed meaning to any other test (antibody, antigenic, molecular) that should refer to that one. This would account for the gigantic incongruities and discordances found in their application, in clinical correlates and in epidemiology, and in the irrationality of the measures.

The only successful isolation was the one of the children, youths, adolescents, adults, seniors, and the whole society, decided on the results and consequences of a thus imposed science.

We believe that an afterthought of the entire matter shall be made mandatory – not the vaccine, with the guidance of the scientific method, the one Galileo Galilei gave an initial serious setting, even if hindered by the Holy Inquisition of that time.

Trieste, 8th September 2021

Aknowledgements: I owe dr Luciano Macrì and dr Roberto Serpieri, engineer, for their very useful comments and corrections.

Annexes: Letter of prof Maria Rosaria Capobianchi + files of publications

Request of withdrawal to Eurosurveillance of the Corman publication

Stefano Scoglio. The final evidence (La prova definitiva), 8th June 2021

References

1 Papadopuols-Eleopulos E. et al., http://www.virusmyth.org/aids/hiv/epreplyek.htm

2 Sinoussi F, Mendiola L, Chermann JC., Purification and partial differentiation of the particles of murine sarcoma virus (M. MSV) according to their sedimentation rates in sucrose density gradients. Spectra 1973; 4:237-243.

3 Letter by Gonda M to Popovic M 26 marzo 1984

MAR 27 REC'D

NATIONAL CANCER INSTITUTE

FREDERICK CANCER RESEARCH FACILITY

P.O. Box B, Frederick, Maryland 21701

March 26, 1984

Dr. Mika Popovic
Laboratory of Tumor Cell Biology
NIH
Building 37, Room 6B22
Bethesda, MD 20205

Dear Mika:

I am sending you 4 extra copies of results requested by Betsy Read. She said Dr. Gallo wanted these micrographs for publication because they contained HTLV particles. If this assumption is based on the cultures being antigen posttive, I would like to point out that the "particles" in micrograph 0905 are in debris of a degenerated cell. No other extracellular "virus-like particles" were observed free between cells anywhere in the pellet. The small extracellular vesicles in 0904 are at least 50% smaller than HTLV mature particles seen in type I, II, or III. Again, these vesicles can be found in any cell pellet. I do not believe any of the particles photographed are HTLV I, II, or III.

Best regards,

Matt

Matthew A. Gonda, Ph.D.
Head, Electron Microscopy Laboratory

MAG:jah

Enclosures

cc: Dr. Gallo
Betsy Read

PRI PROGRAM RESOURCES, INC. • Operations and Technical Support

Enlarged text follows →

Dear Mika:

I am sending you 4 extra copies of results requested by Betsy Read. She said Dr. Gallo wanted these micrographs for publication because they contained HTLV particles. If this assumption is based on the cultures being antigen positive, I would like to point out that the "particles" in micrograph 0905 are in debris of a degenerated cell. No other extracellular "virus-like particles" were observed free between cells anywhere in the pellet. The small extracellular vesicles in 0904 are at least 50% smaller than HTLV mature particles seen in type I, II, or III. Again, these vesicles can be found in any cell pellet. I do not believe any of the particles photographed are HTLV I, II, or III.

Best regards,

Matt

Matthew A. Gonda, Ph.D.

4 Popovic M, Gallo R et al., Science 1984;224:497-500

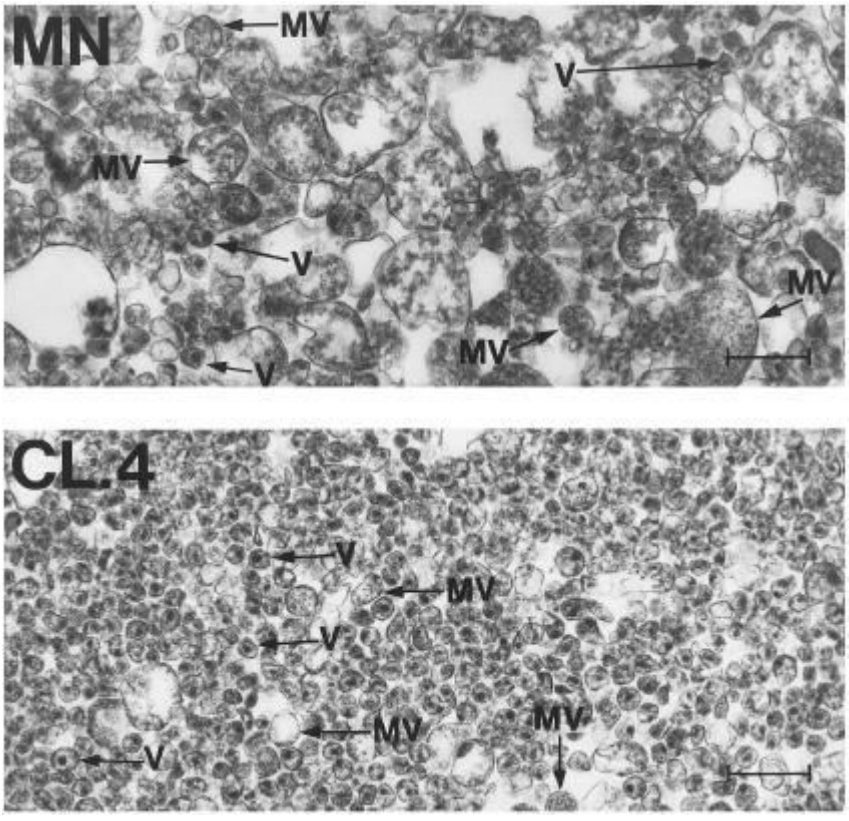
5 Popovic M, Gallo R et al., Science 1984;224:497-500

The yield of virus from H4/HTLV-III cells was assessed by purification of concentrated culture fluids through a sucrose density gradient and assays of particulate RT activity in each fraction collected from the gradient. As shown in Fig. 2b, the highest RT activity was found at a density of 1.16 g/ml, which is similar to other retroviruses. The highest RT activity was found in the fractions with the largest amount of virus, as determined by electron microscopy. The

6 Barré-Sinoussi F et al. Science 1983;220:868

That this new isolate was a retrovirus was further indicated by its density in a sucrose gradient, which was 1.16, and by its labeling with [^{3}H]uridine (Fig. 1). Electron microscopy of the infected umbilical cord lymphocytes showed characteristic immature particles with dense crescent (C-type) budding at the plasma membrane (Fig. 2).

7 "Virus isoled" in density gradient. The viral particles are indicated with "V". Bess GW et al. Virology 230, 134–144 (1997)



8 Guschankof P et al. Virology 230, 125–133 (1997)

9 Christine Maggiore Interview to Eleni Papadopulos-Eleopulos
http://www.virusmyth.com/aids/hiv/cjinterviewep.htm

10 Papadopuolos-Eleopulos E. et al.
http://www.theperthgroup.com/SCIPAPERS/EPEGalloProveRoleHIVEmergMedOCR1993.pdf
Has Gallo proven the role of HIV in AIDS?
11 Papadopuolos-Eleopulos E. et al.
http://www.theperthgroup.com/SCIPAPERS/MHMONT.pdf A critique to Montagnier.
12 Papadopulos-Eleopulos E, Turner VF, Papdimitriou JM. Is a Positive Western Blot Proof of HIV Infection? Bio/Technology 1993;11:696-707.
13 Papadopuolos-Eleopulos E. et al.
http://theperthgroup.com/LATEST/PGRevisitHIVExistence.pdf
14 Papadopuolos-Eleopulos E. et al.
http://www.theperthgroup.com/CONTINUUM/HaemophiliaConn.pdf haemophilia Connection
15 Papadopuolos-Eleopulos E. et al.
http://www.theperthgroup.com/CONTINUUM/PapadopolousReallyAchieved1996.pdf
Isolation of HIV really achieved.
16 Papadopulos-Eleopulos E, Turner VF, Papadimitriou JM, Causer D. HIV antibodies: Further questions and a plea for clarification. Curr. Med. Res. Opin. 1997;13:627-634.
17 http://www.virusmyth.org/aids/hiv/epreplyek.htm isolated facts about HIV a reply
18 Papadopuolos-Eleopulos E. et al.
http://www.theperthgroup.com/CONTINUUM/epeondjamel.html Commentary on Montagnier
19 Djamel Tahi Intervista Luc Montagnier
https://www.virusmyth.com/aids/hiv/dtinterviewlm.htm (annexe)
20 Zhu N et al., N Engl J Med 2020;382:727-33. DOI: 10.1056/NEJMoa2001017
21 Zhou P et al., Nature 2020; 579:270 12 March 2020
22 Prof Ariberto Fassati. MD PhD of the Division of Infection & Immunity, School of Medical Sciences, University College London
23 Gioia Locati http://blog.ilgiornale.it/locati/2020/08/08/il-lockdown-sono-piu-efficaci-disciplina-e-igiene/
24 Torsten Engelbrecht and Konstantin Demeter. COVID19 PCR Tests are Scientifically Meaningless . Bulgarian Pathology Association. https://bit.ly/34U60lA
25 WHO https://www.who.int/news/item/14-12-2020-who-information-notice-for-ivd-users

WHO Information Notice for IVD Users 14 December 2020

Nucleic acid testing (NAT) technologies that use real-time polymerase chain reaction (RT-PCR) for detection of SARS-CoV-2

14 December 2020

"**In some circumstances, the distinction between background noise and actual presence of the target virus is difficult to ascertain.** Thus, the IFU will state how to interpret specimens at or near the limit for PCR positivity. In some cases, the **IFU will state that the cut-off should be manually adjusted to ensure that specimens with high Ct values are not incorrectly assigned SARS-CoV-2 detected due to background noise."**

26 Lavezzo E, Neil M. Ferguson, Dorigatti I, Crisanti Andrea, Imperial College COVID-19 Response Team, et al., Nature https://doi.org/10.1038/s41586-020-2488-1 .
27 Ai T, Yang Z, Hou H, Zhan C, Chen C, Lv W, et al., Correlation of chest CT and RT-PCR testing in coronavirus disease 2019 (COVID-19) in China: a report of 1014 cases. Radiology. February 26, 2020; 1-23.
28 F.Q. Coronavirus, the president of 118: "Cases Covid-like: interstitial penumonia and negative PCR". Pregliasco: "Preoccupano" Il Fatto Quotidiano 17/05/2020.

29 Stefano Scoglio. The final evidence that virus isolation is a charade (La prova definitiva che l'isolamento virale è una farsa) 18th June 2021 (annexe).
30 Amendola A, Capobianchi MR et al., Cardiovascular Research (2021) 117, 1557–1566
31 WHO, 14th December 2020, see previous note.
32 Bustin S and Nolan T. Int. J. Mol. Sci. **2020**, 21, 3004
33 Bustin S and Nolan T Eur J Clin Invest 2017; 47 (10): 756–774
34 Matusali, G. et al., SARS-CoV-2 Serum Neutralization Assay: A Traditional Tool for a Brand-New Virus. Viruses 2021, 13, 655.
35 Chia WN et al., *Lancet Microbe* 2021; 2: e240–49
36 Cristiana Pulcinelli.Maria Capobianchi: the anibody test is still not reliable. https://www.scienzainrete.it/argomenti/covid-19-intervista. Pubblicato il 04/04/2020
37 ADNKRONOS. Interview to prof Maurizio Pregliasco https://www.adnkronos.com/pregliasco-obbligo-vaccinale-per-over-40-o-green-pass-pesante_5y53TOLrsfvuwmY920fs1b 24th August 2021.
38 Giovanni Rezza. **GENERAL DIRECTION OF HEALTHCARE.** Ministry of Health. **Vaccination of the subjects who had SARS-CoV-2 (Vaccinazione dei soggetti che hanno avuto un'infezione da SARS-CoV-2). 3rd March 2021**
39 Ciccosanti F et al., Antiviral Research 190 (2021) 105064
40 Novelli G, Capobianchi MR et al., Cell Death and Disease (2021) 12:310
41 Colavita, F. Capobianchi MR et al., COVID-19 Rapid Antigen Test as Screening Strategy at Points of Entry: Experience in Lazio Region, Central Italy, August–October 2020. Biomolecules 2021, 11, 425.
42 Liotti FM, Capobianchi MR et al., Clinical Microbiology and Infection 27 (2021) 487e488
43 ISTAT Health Ministry. FIRST RESULTS OF THE SEROPREVALENCE SURVEY ON SARS-CoV-2. 3rd August 2020
44 Watson J et al., Interpreting a covid-19 test result. *BMJ* 2020;369:m1808 doi: 10.1136/bmj.m1808 (Published 12 May 2020)
45 Nardacci R, Capobianchi MR et al., Cell Death and Disease (2021) 12:263
46 ICTV Coronaviridae

47 Screen gauge: jruler.exe
48 Goldsmith CS et al., Lancet Vol 395 May 30, 2020.
49 Hartcourt J et al., Emerging Infectious Diseases. www.cdc.gov/eid Vol. 26, No. 6, June 2020
50 Ge et al. Nature 2013; 503:535
51 Bao L et al., Nature 2020; 583:830.
52 Andreano E et al., 2021, Cell 184, 1821–1835.

53 Rondinone, V et al., Viruses 2021, 13,276.
54 Manzulli, V., Capobianchi MR et al. Real Time PCR and Culture-Based Virus Isolation Test in Clinically Recovered Patients: Is the Subject Still Infectious for SARS-CoV2? J. Clin. Med. 2021, 10, 309.
55 Miersch S et al., BioRxiv https://doi.org/10.1101/2020.10.31.362848 ; this version posted December 21st, 2020.
56 Il Piccolo, 17/05/2021

Dal virus al cancro
Il grande successo
dei monoclonali

MAURO GIACCA

Sono più di 20 gli anticorpi monoclonali già sperimentati o in fase di sviluppo contro Covid-19, alcuni dei quali hanno già ricevuto autorizzazione dalle agenzie regolatorie per l'uso di emergenza. Ma la specificità di bersaglio che rende i monoclonali vincenti contro i tumori è anche il loro tallone di Achille nella lotta ai virus, perché questi tendono a cambiare in continuazione, rendendo l'azione dell'anticorpo inefficace (è per questo motivo che il sistema immunitario reagisce alle infezioni virali producendo centinaia di anticorpi diversi contro tanti bersagli del virus anzichè una singola molecola). Tanto che già oggi, visto il successo del vaccino e l'insorgenza delle varianti, molte delle grandi biotec che producono monoclonali stanno interrompendo il loro investimento nel Covid per tornare alla ricerca di molecole sempre più efficaci, specialmente per la terapia dei tumori. —

57 Colavita F et al., INMI COVID-19 Laboratory Team and INMI COVID-19 Study Group. Open Forum Infect Dis. 2020 Sep 2;7(10):ofaa403.
58 Lake MA., What we know so far: COVID-19 current clinical knowledge and research. Clin Med (Lond) 2020; 20:124–7.
Velavan TP, Meyer CG. The COVID-19 epidemic. Trop Med Int Health 2020;25:278–80.
59 Sauvat et al., Cell Death and Disease (2020) 11:656
60 Colavita F et al., Annals of Internal Medicine. doi:10.7326/M20-1176.
61 Capobianchi MR et al., Clinical Microbiology and Infection 26 (2020) 954e956
62 Pieter Borger et al., 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 (annexed document)

La prova definitiva che l'isolamento dei virus è una farsa

Dr. Stefano Scoglio, Ph.D.

Sono partito già dal Marzo 2020 col denunciare che il presunto isolamento del SARS-Cov2, eseguito in primis dall'equipe dell Chinese Center for Disease Control (CCDC) sotto il nome Zhu N. et al., non era affatto un isolamento, perché non c'era nessuna purificazione del virus, ma solo la messa in coltura su cellule di rene di scimmia del liquido bronco-alveolare di alcuni pazienti affetti da polmonite. Come dissi allora, quel liquido bronco-alveolare, più o meno centrifugato, conteneva circa 30 miliardi di particelle simil-virali, la maggior parte dei quali di origine umana (esosomi, vescicole extra-cellulari, etc)., che veniva poi messo in coltura su cellule di rene di scimmia Vero E6.

Uno potrebbe obiettare: ma chi se ne frega se è stato isolato, il virus c'è e ammala. Ma è proprio qui il problema: per poter dire che la causa di una malattia è un virus, e non tanti altri possibili fattori, come quelli alimentari, ambientali e iatrogeni (causati dai farmaci e dalle terapie stesse), occorre prima identificare il virus, il che significa isolarlo/purificarlo estraendolo dalla enorme massa di miliardi di particelle simil-virali presenti nel liquido del paziente; e poi, una volta isolato, verificare che sia patogeno, che possa far ammalare, il che è possibile solo se io testo su una cavia un materiale composto quasi esclusivamente dal virus, perché se anche ci fosse un effetto patogeno, se il materiale da me testato è grandemente eterogeneo, cioè composto di un grande numero di altri possibili fattori, non si potrà mai sapere se quel virus che ipotizzo essere la causa della malattia (in questo caso, Covid) ne sia veramente la causa. In sintesi, questa è l'essenza di quei principi fondamentali della microbiologia che si chiamano i Postulati di Koch.

In miei precedenti scritti (e in maniera ancora più dettagliata nel libro che sto per pubblicare) ho mostrato come tali Postulati di Koch non siano stati minimamente soddisfatti dai ricercatori, e dunque non c'è nessuna possibilità di affermare, con nessun grado neppure di probabilità, che le polmoniti bilaterali interstiziali e le trombo-embolie polmonari, che costituiscono l'essenza della malattia Covid (e che sono sempre esistite, e prima del 2020 si chiamavano col

loro nome proprio) siano causate da un virus, e tantomeno dallo specifico virus SARS-Cov2.

Sono stato attaccato anche duramente per questa mia posizione, tacciata come negazionista, ma i veri negazionisti sono coloro che negano la vera scienza, volendo far passare per certo e provato solo ciò che è una mera ipotesi. Oggi, la mia posizione è definitivamente confermata da uno dei più importanti organi della sanità *mainstream* mondiale, il Center for Disease Control, o CDC, americano.

Dopo la comparsa della discussione sul presunto virus, già nel 2020 sono iniziate ad accadere cose strane. Nell'Aprile 2020, la Commissione Europea rilascia la seguente dichiarazione:

> "Since no virus isolates with a quantified amount of the SARS-CoV-2 are currently available…".[1]
>
> "Poiché nessun isolato con un ammontare quantificato di SARS-Cov2 è attualmente disponibile…".

E qualche tempo dopo, nel Luglio 2020, la stessa cosa viene ripetuta dal CDC americano:

> "Since no quantified virus isolates of the 2019-nCoV are currently available…".[2]
>
> "Poiché nessun isolato virale quantificato è attualmente disponibile".

Utilizzai l'affermazione per mostrare come il non isolamento del virus fosse confermato anche dalle principali istituzioni. E tuttavia, la dichiarazione era strana, perché, anche se si affermava che non esisteva nessuna quantificazione del virus, si parlava comunque ancora di "isolati".

La stranezza sta nel fatto che, a rigor di logica, un isolato è intrinsecamente quantificato: isolamento significa separazione di un qualsiasi materiale, molecola o organismo dall'intero complesso di cui fa parte; pertanto, idealmente l'isolato

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[1] European Commission, Working Document of Commission Services, Current performance of COVID-19 test methods and devices and proposed performance criteria, April 16 2020, p.19.

[2] Center for Disease Control and Prevention, Division of Viral Diseases, CDC 2019-Novel Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel, 13/07/2020, p.39).

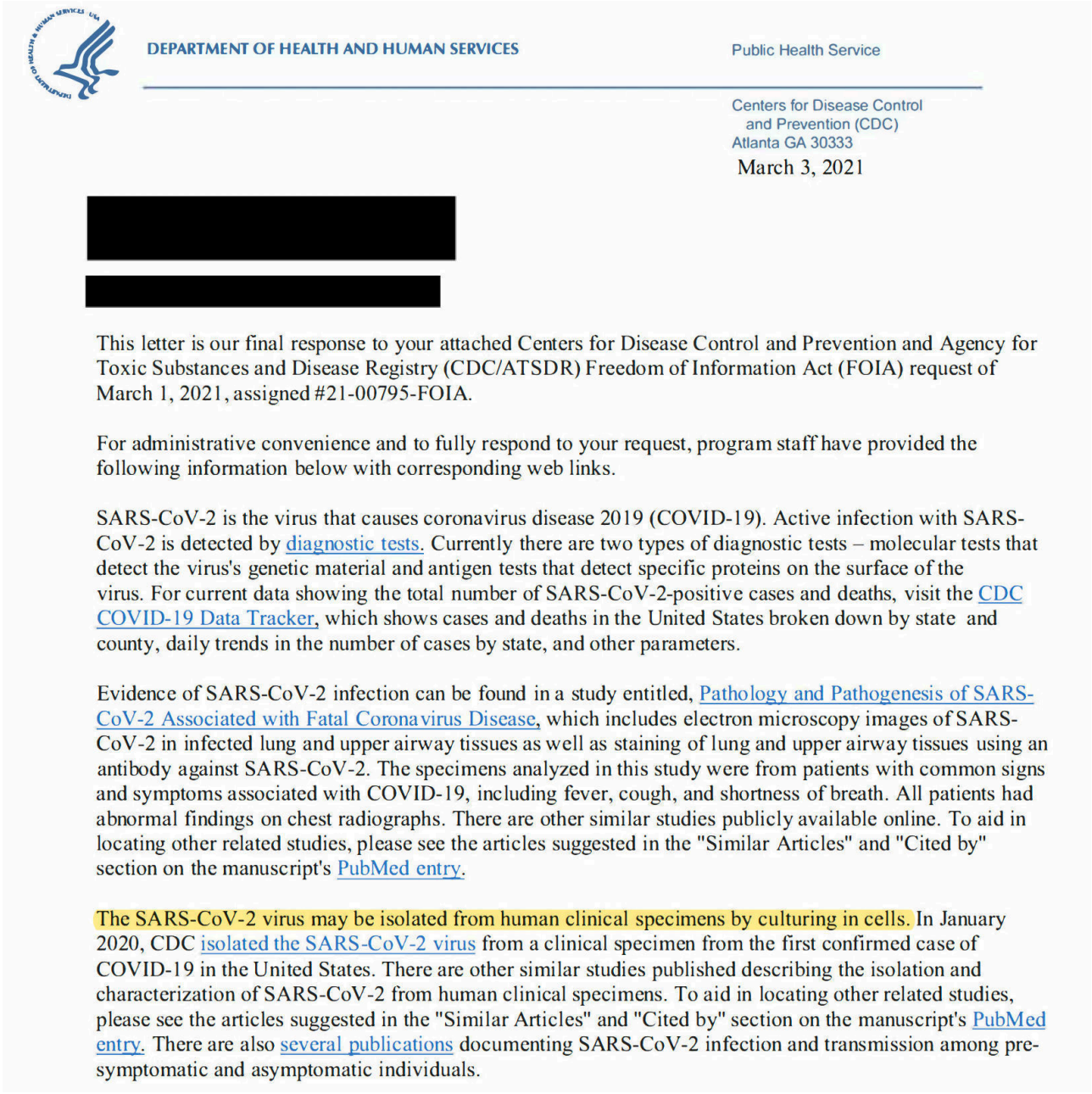
costituisce il 100% del nuovo materiale isolato che si ottiene. Può darsi che non si possa raggiungere il 100% per la presenza di qualche impurità, ma comunque si parlerebbe di un isolato al +/- 95%. Questo non sarebbe ideale, perché se io devo essere certo che un certo batterio o "virus" sia patogeno, ne devo testare la patogenicità nel suo stato di isolato puro, o mi resta sempre il dubbio che l'eventuale effetto patogeno possa essere dovuto alle impurità presenti. Ma potrei almeno parlare di una probabilità molto elevata, al 95%.

La principale obiezione dei virologi a realizzare questi isolati purificati è che i virus non possono sussistere al di fuori delle cellule ospiti, e quindi non si possono "isolare" se non attraverso delle colture cellulari. Si tratta di un'obiezione infondata: il presunto virus non è un organismo vivente, quindi non può morire, è una molecola, e dunque se isolato, per quanto non proliferi, mantiene la sua struttura, è può dunque riattivarsi una volta messo su altre cellule. E questo consentirebbe di definire il virus, sequenziarne il genoma in modo corretto, e a quel punto ritrovarlo e quantificarlo nelle colture cellulari in cui lo si pone dopo averlo isolato. Senza nessun previo isolamento, la messa in coltura è messa in coltura di Dio solo sa cosa!

Anche volendo adeguarsi alla modifica dei postulati di Koch effettuata da Rivers nel 1937, si può anche ammettere che, per le prove di patogenicità, si utilizzino non il virus isolato ma le colture cellulari in cui si farebbe proliferare il virus, ma per poter avere la certezza che quelle sono colture cellulari di uno specifico virus, occorre prima conoscere il virus, che dunque deve essere preventivamente isolato/purificato.

Insomma, senza previo isolamento/purificazione del virus tutto ciò che ne ne consegue non ha alcun senso. Ecco perché affermare di aver prodotto un isolato non quantificato non ha alcun senso, è una contraddizione in termini. Contraddizione che esplode in tutta la sua gravità in un recente documento ufficiale dello stesso CDC.

Il CDC americano ha risposto a due richieste sull'isolamento del virus avanzate sulla base del Freedom of Information Act (FOIA). Questa è la risposta alla prima:



DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

March 3, 2021

[redacted]

This letter is our final response to your attached Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) Freedom of Information Act (FOIA) request of March 1, 2021, assigned #21-00795-FOIA.

For administrative convenience and to fully respond to your request, program staff have provided the following information below with corresponding web links.

SARS-CoV-2 is the virus that causes coronavirus disease 2019 (COVID-19). Active infection with SARS-CoV-2 is detected by diagnostic tests. Currently there are two types of diagnostic tests – molecular tests that detect the virus's genetic material and antigen tests that detect specific proteins on the surface of the virus. For current data showing the total number of SARS-CoV-2-positive cases and deaths, visit the CDC COVID-19 Data Tracker, which shows cases and deaths in the United States broken down by state and county, daily trends in the number of cases by state, and other parameters.

Evidence of SARS-CoV-2 infection can be found in a study entitled, Pathology and Pathogenesis of SARS-CoV-2 Associated with Fatal Coronavirus Disease, which includes electron microscopy images of SARS-CoV-2 in infected lung and upper airway tissues as well as staining of lung and upper airway tissues using an antibody against SARS-CoV-2. The specimens analyzed in this study were from patients with common signs and symptoms associated with COVID-19, including fever, cough, and shortness of breath. All patients had abnormal findings on chest radiographs. There are other similar studies publicly available online. To aid in locating other related studies, please see the articles suggested in the "Similar Articles" and "Cited by" section on the manuscript's PubMed entry.

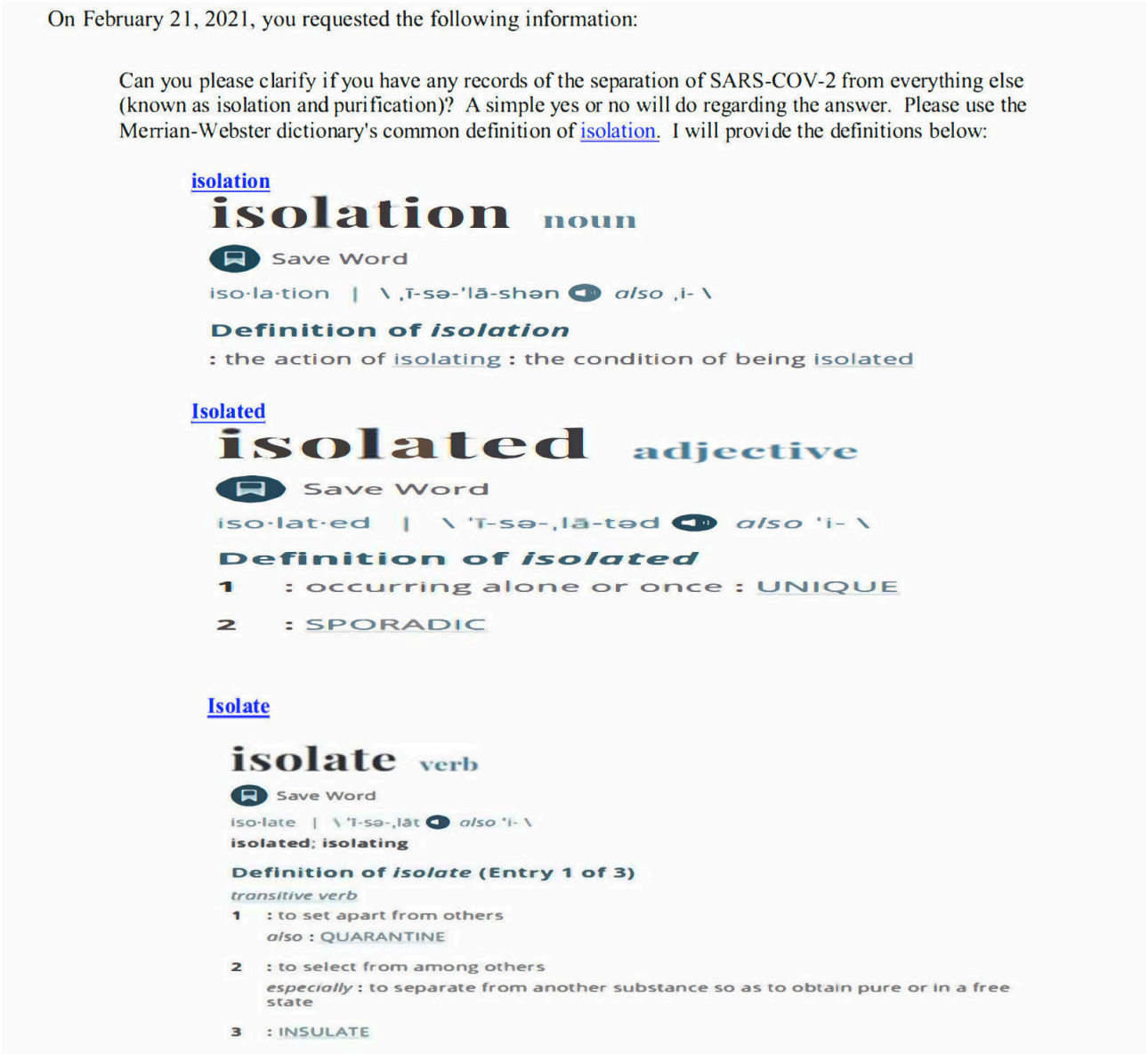
The SARS-CoV-2 virus may be isolated from human clinical specimens by culturing in cells. In January 2020, CDC isolated the SARS-CoV-2 virus from a clinical specimen from the first confirmed case of COVID-19 in the United States. There are other similar studies published describing the isolation and characterization of SARS-CoV-2 from human clinical specimens. To aid in locating other related studies, please see the articles suggested in the "Similar Articles" and "Cited by" section on the manuscript's PubMed entry. There are also several publications documenting SARS-CoV-2 infection and transmission among pre-symptomatic and asymptomatic individuals.

Qui, la frase chiave è:

> “The SARS-Cov2 virus may be isolated from human clinical specimens by culturing in cells.”
>
> “Il virus SARS-Cov2 può essere isolato da campioni umani clinici coltivandolo in coltura cellulare.”

Questo conferma quello che sospettavamo, e che sono andato ripetendo in questi ultimi mesi: laddove l’isolamento è un procedimento di sottrazione, ovvero tu sottrai ciò che vuoi isolare dal complesso di cui fa parte, qui l’isolamento viene identificato con un procedimento moltiplicativo, la messa in coltura, che è l’esatto opposto dell’isolamento.

In una seconda richiesta FOIA, questo elemento è stato ulteriormente specificato, perché chi ha sottoposto la richiesta ha addirittura riportato la definizione di isolamento del vocabolario proprio per evitare che si giocasse sulla terminologia:



On February 21, 2021, you requested the following information:

Can you please clarify if you have any records of the separation of SARS-COV-2 from everything else (known as isolation and purification)? A simple yes or no will do regarding the answer. Please use the Merrian-Webster dictionary's common definition of isolation. I will provide the definitions below:

isolation

isolation noun

Save Word

iso·la·tion | \ ˌī-sə-ˈlā-shən *also* ˌi- \

Definition of *isolation*

: the action of isolating : the condition of being isolated

Isolated

isolated adjective

Save Word

iso·lat·ed | \ ˈī-sə-ˌlā-təd *also* ˈi- \

Definition of *isolated*

1 : occurring alone or once : UNIQUE

2 : SPORADIC

Isolate

isolate verb

Save Word

iso·late | \ ˈī-sə-ˌlāt *also* ˈi- \

isolated; isolating

Definition of *isolate* (Entry 1 of 3)

transitive verb

1 : to set apart from others
also : QUARANTINE

2 : to select from among others
especially : to separate from another substance so as to obtain pure or in a free state

3 : INSULATE

Quindi, la richiesta è specifica, e si chiede se il virus è stato isolato secondo la definizione comune di "isolamento", come riportata nel vocabolario:

"to set apart from others" - "Separare dagli altri";

"Select among others - to separate from another substance so as to obtain pure or in a free state" -

"Selezionare tra gli altri - separare da un'altra sostanza in modo da ottenere un elemento puro o in uno stato libero."

A questo punto la richiesta è ineludibile, e questa è la sorprendente riposta del CDC (il documento completo è allegato in appendice):

Isolate

isolate verb

Save Word

iso·late | \ ˈī-sə-ˌlāt also ˈi- \

isolated; isolating

Definition of *isolate* (Entry 1 of 3)

transitive verb

1 : to set apart from others
also : QUARANTINE

2 : to select from among others
especially : to separate from another substance so as to obtain pure or in a free state

3 : INSULATE

The SME states the following:

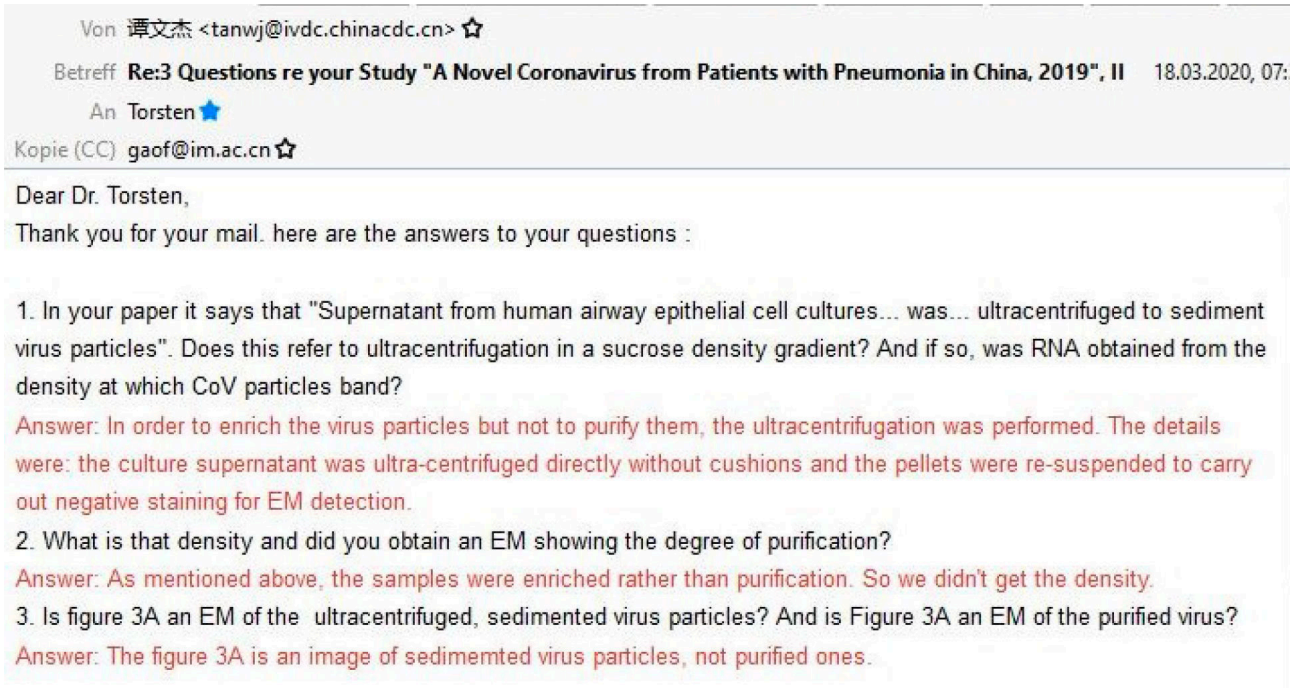
The definition of "isolation" provided in the request is outside of what is possible in virology, as viruses need cells to replicate, and cells require liquid food. However, the SARS-CoV-2 virus may be isolated from a human clinical specimen by culturing in cell culture, which is the definition of "isolation" as used in microbiology, and as indicated in the previous round of response in the resources provided.

"**La definizione di "isolamento" fornita nella richiesta è al di fuori di ciò che è possibile in virologia**, dato che i virus hanno bisogno delle cellule per replicarsi, e le cellule hanno bisogno di cibo liquido. Tuttavia, il virus SARS-Cov2 può essere **isolato da un campione clinico umano mettendolo in coltura cellulare**, che è la **definizione di isolamento utilizzata in microbiologia…**"

Quindi, quando i virologi dicono che hanno isolato un virus, non intendono dire che l'hanno purificato, separato dal resto del materiale organico in cui si trova. No, intendono l'opposto, ovvero per loro isolare significa moltiplicare, cercare di far proliferare, l'esatto contrario del significato del termine "isolamento".

Ad esempio, questa è la risposta degli scienziati cinesi dell'equipe che, per la prima volta al mondo hanno detto di aver isolato il SARS_Cov2[3], ad una richiesta di chiarimento avanzata dal mio amico e giornalista tedesco Torsten Engelbrecht:

[3] Zhu N et al, A Novel Coronavirus from Patients with Pneumonia in China, 2019, N Engl J Med. 2020 Feb 20; 382(8): 727–733.



Von 谭文杰 <tanwj@ivdc.chinacdc.cn>

Betreff **Re:3 Questions re your Study "A Novel Coronavirus from Patients with Pneumonia in China, 2019", II** 18.03.2020, 07:

An Torsten

Kopie (CC) gaof@im.ac.cn

Dear Dr. Torsten,

Thank you for your mail. here are the answers to your questions :

1. In your paper it says that "Supernatant from human airway epithelial cell cultures... was... ultracentrifuged to sediment virus particles". Does this refer to ultracentrifugation in a sucrose density gradient? And if so, was RNA obtained from the density at which CoV particles band?

Answer: In order to enrich the virus particles but not to purify them, the ultracentrifugation was performed. The details were: the culture supernatant was ultra-centrifuged directly without cushions and the pellets were re-suspended to carry out negative staining for EM detection.

2. What is that density and did you obtain an EM showing the degree of purification?

Answer: As mentioned above, the samples were enriched rather than purification. So we didn't get the density.

3. Is figure 3A an EM of the ultracentrifuged, sedimented virus particles? And is Figure 3A an EM of the purified virus?

Answer: The figure 3A is an image of sedimemted virus particles, not purified ones.

Alla domanda se l'ultra-centrifugazione del campione biologico dei pazienti effettuata dai ricercatori cinesi fosse stata fatta in gradiente di densità (una tecnica usata per la purificazione di material biologico), i ricercatori rispondono:

"Come detto sopra, i campioni sono stati arricchiti piuttosto che purificati…"

Questo conferma quello che ho detto sopra: il processo normalmente utilizzato in virologia non purifica, ovvero non sottrae, ma arricchisce, ovvero moltiplica il già super-complesso secreto del paziente in una coltura cellulare altrettanto complessa, dato che le stesse cellule di rene di scimmia hanno la stessa complessità genica e molecolare delle cellule umane del paziente.

La dichiarazione del CDC vista sopra rappresenta una conferma eclatante e a queso punto indiscutibile: **i virus non possono essere isolati**, non nel senso corretto del termine, perché ciò è "…al di fuori di ciò che è possibile in virologia".

Abbiamo già risposto alla misera scusa con cui il CDC giustifica questa impossibilità a isolare, secondo cui i virus hanno bisogno delle cellule per

replicarsi, ma ripetiamo : il CDC afferma che i virus hanno bisogno delle cellule per "replicarsi", non per sopravvivere, proprio perché il virus, non essendo un organismo vivente, non può morire, è una molecola di acido nucleico in una capsula lipoproteica. In quanto tale, il presunto virus può essere isolato come qualsiasi altra molecola, e come per tutte le molecole la loro attività è data dalla loro struttura. Quindi, isolando un presunto virus integro, che mantiene la sua struttura, dopo averlo purificato e analizzato, lo si può mettere in coltura su cellule sane, e usare quella coltura per le prove di patogenicità.

La cosa sorprendente è che gli esosomi, che sono indistinguibili dai virus e hanno la stessa dimensione e struttura dei presunti virus[4], sono invece isolati in modo corretto.[5] E allora perché i virologi non fanno lo stesso? Forse perché dovrebbero ammettere che cercando di isolare potenziali virus super-tossici in realtà non fanno che isolare innocui esosomi? Questo porterebbe a prove di patogenicità in cui la tossicità e l'effetto patogeno sarebbe del tutto assente, e questo porrebbe in una crisi esiziale le stesse fondazioni della virologia.

E così, i virologi si ostinano a generare colture indistinte, senza nessuna conoscenza preliminare del virus che si vuole testare, con prove di patogenicità del tutto manipolate e truccate.

I virologi affermano che c'è un virus patogeno nella coltura cellulare perché le cellule Vero (di rene di scimmia), su cui viene immesso l'estratto di secreto del paziente, dopo 3 o 5 gg iniziano a morire. Questa sarebbe la prova, senza nessun preliminare isolamento del virus, che nel secreto del paziente si ha un virus patogeno che uccide le cellule Vero. Ma soprattutto, tutte le volte che vien fatto questo esperimento di "isolamento virologico" attraverso la prova degli effetti citopatici (patogenicità cellulare) su cellule Vero, i virologi non si preoccupano mai di fare un test di controllo adeguato e corretto, per verificare cosa succederebbe alle stesse cellule Vero senza l'immissione di nessun liquido del paziente.

A volte il controllo viene fatto, ma in modo manipolatorio: come sottolineai in un articolo scritto sul presunto primo isolamento del virus da parte dell'equipe

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[4] Giannessi F et al., The Role of Extracellular Vesicles as Allies of HIV, HCV and SARS Viruses, Viruses 2020, 12, 571; pp. 572-4.

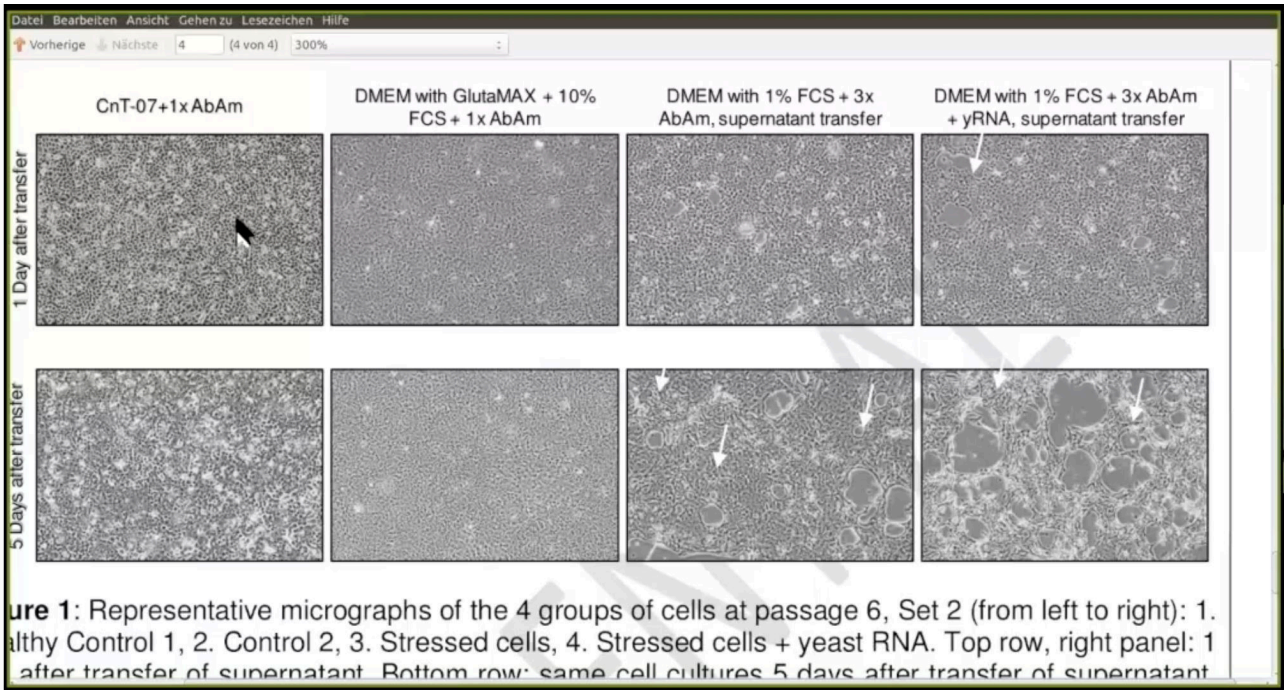
[5] Li P. et al., Progress in Exosome Isolation Techniques, Theranostics. 2017; 7(3): 789–804.

cinese di Zhu et al.[6], i ricercatori cinesi fecero la solita coltura cellulare e trovarono che dopo 4 gg le cellule Vero iniziavano a morire; mentre nel controllo, ovvero senza nessuna immissione di materiale presuntivamente infetto, accadde la stessa cosa, ma in 6 gg. Questo fu interpretato come indice del fatto che nella coltura dove fu immesso materiale presuntivamente infetto c'era il virus! Ma a parte che una differenza di 2 gg non sembra sufficiente a trarre nessuna conclusione, gli autori nascosero il fatto che le due colture erano differenti: quelle col "virus" erano cellule di cancro al polmone, mentre quelle del controllo erano cellule Vero di rene di scimmia, che sono chiaramente più "robuste" e meno fragili di quelle tumorali. Era quindi chiaro che i dati non avevano nessun valore. Ma in generale, neppure un tale finto controllo viene eseguito.

Le cellule di rene di scimmia sono sottoposte al test di cito-patogenicità non in uno stato neutro, ma con l'aggiunta di antibiotici, ormoni e altri nutrienti sintetici; e dato che tali ingredienti sono anch'essi relativamente tossici, per confermare che la tossicità cellulare sia dovuta al virus e non ad altro, occorre verificare in parallelo che la mistura di cellule Vero non degradi e non produca effetti auto-tossici di per sé, senza l'intervento di nessun secreto di paziente. Questo, però, non viene mai fatto.

Lo ha fatto, recentemente, l'equipe del dr. Stefan Lanka, che non ha ancora completato lo studio, mancando le fasi del passaggio al microscopio elettronico, e del sequenziamento, ma ha diffuso i primi risultati, già estremamente significativi.

[6] Zhu N et al, A Novel Coronavirus from Patients with Pneumonia in China, 2019, N Engl J Med. 2020 Feb 20; 382(8): 727–733.



ure 1: Representative micrographs of the 4 groups of cells at passage 6, Set 2 (from left to right): 1. althy Control 1, 2. Control 2, 3. Stressed cells, 4. Stressed cells + yeast RNA. Top row, right panel: 1 after transfer of supernatant. Bottom row: same cell cultures 5 days after transfer of supernatant

Qui sopra si vedono le diapositive delle colture cellulari sviluppate dall'equipe del Dr. Lanka, **senza l'aggiunta di nessun secreto di pazienti presuntivamente affetti da una patologia virale**, ma seguendo la procedura normalmente usata dagli stessi virologi per la coltura cellulare del presunto virus. Questa, ad esempio, è la procedura descritta dal gruppo di ricercatori del CDC americano per l'isolamento del SARS-Cov2:

> "Sono stati raccolti campioni clinici da un paziente che aveva acquisito il COVID-19 durante un viaggio in Cina e che è stato identificato a Washington, USA ... I campioni di tampone nasofaringeo (NP) e orofaringeo (OP) sono stati raccolti il terzo giorno dopo l'insorgenza dei sintomi, posti in 2-3 ml di terreno di trasporto virale, utilizzati per la diagnosi molecolare e congelati. I campioni confermati positivi alla PCR sono stati aliquotati e ricongelati fino all'inizio dell'isolamento del virus ... **Abbiamo utilizzato cellule Vero CCL-81 per l'isolamento**...**Abbiamo coltivato cellule Vero E6**, Vero CCL-81, HUH 7.0, 293T, A549 e EFKB3 in Dulbecco minimal essential medium (DMEM) integrato con siero bovino fetale inattivato al calore (5% o 10%) e antibiotici / antimicotici ... Abbiamo quindi tripsinizzato e risospeso cellule Vero in DMEM contenente il 10% di siero bovino fetale, 2x di penicillina / streptomicina, 2x di antibiotici / antimicotici e 2x di amfotericina B a una concentrazione di 2.5 x 10^5 cellule/ml ... Abbiamo quindi fatto crescere le colture inoculate in un incubatore umidificato a 37° C in un'atmosfera al 5% di CO e osservato giornalmente gli **effetti citopatici (CPE**) ... Quando si sono trovati CPE... abbiamo usato 50 µL di lisato virale per l'estrazione dell'acido nucleico totale per i test di conferma e sequenziamento "[7]

[7] Harcourt J et al., Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States, Emerg. Infect. Dis., Volume 26, Number 6, June 2020.

Qui si conferma di nuovo che l'isolamento corrisponde al suo contrario, alla messa in coltura, messa in coltura che viene fatta nel modo descritto, su cellule Vero E6, che però non sono in uno stato puro, ma miscelate con diversi ingredienti: 3 antibiotici, che vengono raddoppiati o triplicati tra la prima e la seconda fase, e che, come dice il termine stesse, sono ingredienti "anti-vita".

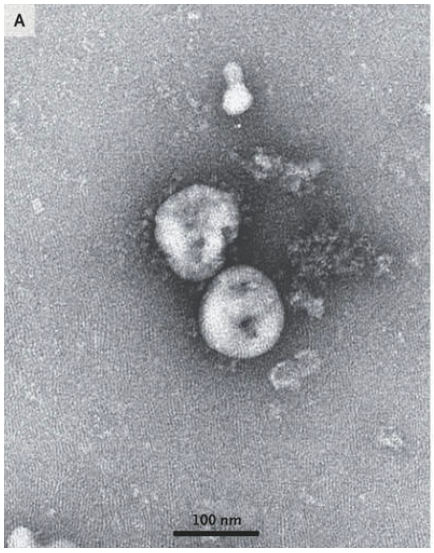
Le diapositive del dr. Lanka mostrano nella banda superiore 4 stadi di trattamento delle cellule Vero al giorno 1, e nella banda sottostante gli stessi 4 stadi al giorno 5. I 4 stadi della procedura sono gli stessi utilizzati in virologia, e simili a quelli descritti nell'articolo del CDC riportato sopra, con l'unica differenza che in questo caso non c'è l'aggiunta di nessun secreto di paziente Covid: al giorno 1, si parte con una coltura di cellule Vero con una piccola quantità di antibiotico; al secondo stadio di aggiunge alla cultura un mix di nutrienti e base di glutammina + siero bovino; al terzo stadio si raddoppia/triplica l'antibiotico, e con questa aggiunta già al primo giorno si notano effetti di degenerazione cellulare; che si aggravano ulteriormente quando si aggiunge anche materiale genetico di sintesi. Agli stadi 3 e 4, dopo 5 gg, senza che sia stato immesso nessun secreto o liquido di paziente presuntivamente patogeno, le cellule decadono nello stesso stato di degenerazione (cito-patogenicità) che si ha quando si aggiunge il secreto "patogeno".

Questo dimostra che l'effetto citotossico non è dovuto a nessun virus patogeno presente nel secreto di un paziente, ma avviene spontaneamente per il modo in cui è strutturata la coltura cellulare. È chiaro, quindi, perché i virologi non fanno mai questo tipo di controllo, perché dovrebbero confessare che il secreto pieno di presunti virus non produce nessuna tossicità ed effetto patogeno ulteriore rispetto a quella che si ha normalmente nella cultura cellulare in sé e per sé.

Questa è dunque la conferma definitiva, oltre alla confessione del CDC, che nessun virus SARS-Cov2 è stato isolato, e di nessun virus si è veramente provata la patogenicità.

C'è un ultima frontiera a cui si possono aggrappare i virologi, quella del microscopio elettronico. I ricercatori dell'equipe di Zhu et al., rispondendo alla richiesta di Torsten Engelbrecht e affermando che non hanno purificato ma invece arricchito il presunto virus, affermano implicitamente che comunque l'esistenza del virus è provata dalle fotografie al Microscopio Elettronico (EM), e che le

preparazioni del campione hanno come scopo proprio la messa a punto per l'analisi EM. Questo è il risultato che loro citano, specificando che non si tratta di "particelle virali sedimentate, non purificate":



Ma senza avere prima isolato e analizzato il virus, come fanno a sapere che quelle viste al microscopio elettronico sono immagini appartenenti al virus che cercano, e non a qualche altro organismo, incluso l'organismo umano, visto che è noto che i secreti di pazienti umani contengono particelle geniche umane (vescicole extracellulari, esosomi, etc.) fino al 95% del materiale?[8] Non lo sanno, è solo una ipotesi fatta diventare certezza, e che nasconde completamente il fatto che esistono fotografie al microscopio elettronico di esosomi che appaiono del tutto uguali a quelle attribuite ai coronavirus:

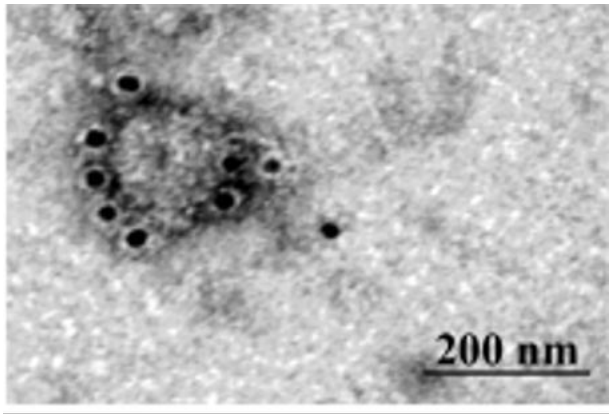


Foto EM di esosoma

[8] Takeuchi S. et al., Metagenomic analysis using next-generation sequencing of pathogens in bronchoalveolar with respiratory failure, in Nature, SCIENTIFIC REPORTS (2019) 9:12909

APPENDICE -
LA LETTERA DI RISPOSTA UFFICIALE
FIRMATA DEL CDC AMERICANO

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333

March 1, 2021

SENT VIA EMAIL

[redacted]

This letter is in response to your February 21, 2021, email regarding our response dated February 21, 2021, to your Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) Freedom of Information Act (FOIA) request of January 6, 2021, assigned #21-00464-FOIA, for the following information:

> All records in the possession, custody or control of CDC/ATSDR describing the isolation of a SARS-COV-2 virus, directly from a sample taken from a diseased patient, where the patient sample was not first combined with any other source of genetic material (i.e. monkey kidney cells aka vero cells; lung cells from a lung cancer patient).
>
> Please note that I am using 'isolation' in the every-day sense of the word: the act of separating a thing(s) from everything else. I am not requesting records where 'isolation of SARS-COV-2' refers instead to:
>
> • the culturing of something, or
> • the performance of an amplification test (i.e. a PCR test), or
> • the sequencing of something.
>
> Please also note that my request is not limited to records that were authored by CDC/ATSDR or that pertain to work done by CDC/ATSDR. My request includes any sort of record, for example (but not limited to) any published peer-reviewed study that CDC/ATSDR has downloaded or printed.
>
> If any records match the above description of requested records and are currently available to the public elsewhere, please provide enough information about each record so that I may identify and access each record with certainty (i.e. title, author(s), date, journal, where the public may access it).

We received your clarification scope dated January 11, 2021, which provided the following information:

> This is not a complex question. I have already received a response from the CDC on this topic in November. The ONLY reason I have resubmitted is because I inquired with LaShanda (LSchofield@cdc.gov) who was my previous case manager. She advised that I resubmit my question due to the following claim by the CDC:
>
> https://www.cdc.gov/coronavirus/2019-ncov/lab/grows-virus-cell-culture.html

For information about the SARS-CoV-2 genome sequence, see the NIH GenBank website (https://www.ncbi.nlm.nih.gov/genbank/sars-cov-2-seqs/), which includes over 44,000 sequences as of December 7, 2020.

On February 21, 2021, you requested the following information:

> Can you please clarify if you have any records of the separation of SARS-COV-2 from everything else (known as isolation and purification)? A simple yes or no will do regarding the answer. Please use the Merrian-Webster dictionary's common definition of isolation. I will provide the definitions below:

isolation

isolation noun

Save Word

iso·la·tion | \ ˌī-sə-ˈlā-shən *also* ˌi- \

Definition of *isolation*

: the action of isolating : the condition of being isolated

Isolated

isolated adjective

Save Word

iso·lat·ed | \ ˈī-sə-ˌlā-təd *also* ˈi- \

Definition of *isolated*

1 : occurring alone or once : UNIQUE

2 : SPORADIC

Isolate

isolate verb

Save Word

iso·late | \ ˈī-sə-ˌlāt *also* ˈi- \

isolated; isolating

Definition of *isolate* (Entry 1 of 3)

transitive verb

1 : to set apart from others
also : QUARANTINE

2 : to select from among others
especially : to separate from another substance so as to obtain pure or in a free state

3 : INSULATE

The SME states the following:

The definition of "isolation" provided in the request is outside of what is possible in virology, as viruses need cells to replicate, and cells require liquid food. However, the SARS-CoV-2 virus may be isolated from a human clinical specimen by culturing in cell culture, which is the definition of "isolation" as used in microbiology, and as indicated in the previous round of response in the resources provided.

> Since the above article is dated December and I received a response in Nov, then there should only be the analysis of the content on that page.
>
> Therefore, I am rejecting the 'complicated' claim and expect a response within 30 business days. If not, I will submit with the Ombudsman right away.

You provided us the following written summary dated February 2, 2021:

> I will respond fully to the FOIA response in this email. I don't remember exactly what I said in my voicemail so I will articulate the entire issue here.
>
> **Summary**
>
> In this section I will summarize my points. Sections after this summary are just my detailed analysis of the references in the 21-00464-FOIA response.
>
> - My FOIA requests the real isolation (separation of SARS-COV-2 from everything else also known as purification) and has not been answered by 21-00464-FOIA
> - 21-00464-FOIA has requested all records that demonstrate the isolation (separation / purification) of SARS-COV-2 since Nov 2020
> - The response to 21-00464-FOIA did not produce any records for the isolation (separation / purification) of SARS-COV-2
> - I am seeking a new response to my initial inquiry of the isolation (separation /purification of SARS-COV-2 between Nov 2020 and present.
> - I do not want any records that do not match my initial request (See attached.).

On February 21, 2021, the subject matter expert (SME) stated the following:

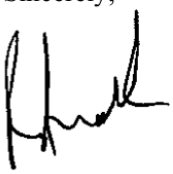
The requester specifies that the requester would like documents related to isolation, defined by the requester as "separation of SARS-COV-2 from everything else also known as purification"; viruses need cells to replicate, and cells require liquid food, so this specific component of the request is outside of what is possible in virology. However, the SARS-CoV-2 virus may be isolated from a human clinical specimen by culturing in cell culture, as indicated in the previous round of response and produced below.

Evidence of SARS-CoV-2 infection can be found in a study entitled, Pathology and Pathogenesis of SARS-CoV-2 Associated with Fatal Coronavirus Disease, which includes electron microscopy images of SARS-CoV-2 in infected lung and upper airway tissues as well as staining of lung and upper airway tissues using an antibody against SARS-CoV-2. The specimens analyzed in this study were from patients with common signs and symptoms associated with COVID-19, including fever, cough, and shortness of breath. All patients had abnormal findings on chest radiographs. There are other similar studies publicly available online. To aid in locating other related studies, please see the articles suggested in the "Similar Articles" and "Cited by" section on the manuscript's PubMed entry (https://pubmed.ncbi.nlm.nih.gov/32437316/).

The SARS-CoV-2 virus may be isolated from human clinical specimens by culturing in cells. In January 2020, CDC isolated the SARS-CoV-2 virus (https://wwwnc.cdc.gov/eid/article/26/6/20-0516_article) from a clinical specimen from the first confirmed case of COVID-19 in the United States. There are other similar studies published describing the isolation and characterization of SARS-CoV-2 from human clinical specimens. To aid in locating other related studies, please see the articles suggested in the "Similar Articles" and "Cited by" section on the manuscript's PubMed entry (https://pubmed.ncbi.nlm.nih.gov/32160149/) . There are also several publications documenting SARS-CoV-2 infection and transmission among pre-symptomatic and asymptomatic individuals (https://pubmed.ncbi.nlm.nih.gov/32364890/).

If you need any further assistance or would like to discuss any additional aspect of the records provided please contact either our FOIA Requester Service Center at 770-488-6399 or our FOIA Public Liaison at 770-488-6277.

Sincerely,



Roger Andoh
CDC/ATSDR FOIA Officer
Office of the Chief Operating Officer
Phone: (770) 488-6399
Fax: (404) 235-1852

21-00464-FOIA

VIRUSMYTH HOMEPAGE <http://www.virusmyth.com/aids/index.htm>

INTERVIEW LUC MONTAGNIER
Did Luc Montagnier Discover HIV?

By Djamel Tahi

/Continuum/ Winter 1997

Text of a videotape interview performed at the Pasteur Institute, July 1997. Please note: The answers by Luc Montagnier have been numbered for easier reference to the analyses in the reply by Papadopulos-Eleopulos et al.
<http://www.virusmyth.com/aids/hiv/epreplyintervlm.htm>

/DT: A group of scientists from Australia argues that nobody up till now has isolated the AIDS virus, HIV. For them the rules of retrovirus isolation have not been carefully respected for HIV. These rules are: culture, purification of the material by ultracentrifugation, Electron Microscopic (EM) photographs of the material which bands at the retrovirus density, characterisation of these particles, proof of the infectivity of the particles. /

LM: No, that is not isolation. We did isolation because we "passed on" the virus, we made a culture of the virus. For example Gallo said : "They have not isolated the virus...and we (Gallo et al.), we have made it emerge in abundance in an immortal cell line." But before making it emerge in immortal cell lines, we made it emerge in cultures of normal lymphocytes from a blood donor. That is the principal criterion. One had something one could pass on serially, that one could maintain. And characterised as a retrovirus not only by its visual properties, but also biochemically, RT [reverse transcriptase] activity which is truly specific of retroviruses. We also had the reactions of antibodies against some proteins, probably the internal proteins. I say probably by analogy with knowledge of other retroviruses. One could not have isolated this retrovirus without knowledge of other retroviruses, that's obvious. But I believe we have answered the criteria of isolation. Totally. (1)

/DT: Let me come back on the rules of retrovirus isolation which are : culture, purification at the density of retroviruses, EM photographs of the material at the retrovirus density, characterisation of the particles, proof of the infectivity of the particles. Have all these steps been done for the isolation of HIV? I'd like to add, according to several published references cited by the Australian group, RT is not specific to retroviruses and, moreover, your work to detect RT was not done on the purified material?/

LM: I believe we published in Science (May 1983) a gradient which showed that the RT had exactly the density of 1.16. So one had a peak which was RT. So one has fulfiled this criterion for purification. But to pass it on serially is difficult because when you put the material in purification, into a gradient, retroviruses are very fragile, so they break each other and greatly lose their infectivity. But I think even so we were able to keep a little of their infectivity. But it was not as easy as one does it today,

because the quantities of virus were nonetheless very weak. At the beginning we stumbled on a virus which did not kill cells. The virus came from an asymptomatic patient and so was classified amongst the non-syncythia-forming, non-cytopathogenic viruses using the co-receptor ccr5. It was the first BRU virus. One had very little of it, and one could not pass it on in an immortal cell line. We tried for some months, we didn't succeed. We succeeded very easily with the second strain. But there lies the quite mysterious problem of the contamination of that second strain by the first. That was LAI. (2)

/DT: Why do the EM photographs published by you, come from the culture and not from the purification?/

LM: There was so little production of virus it was impossible to see what might be in a concentrate of virus from a gradient. There was not enough virus to do that. Of course one looked for it, one looked for it in the tissues at the start, likewise in the biopsy. We saw some particles but they did not have the morphology typical of retroviruses. They were very different. Relatively different. So with the culture it took many hours to find the first pictures. It was a Roman effort! It's easy to criticise after the event. What we did not have, and I have always recognised it, was that it was truly the cause of AIDS. (3)

/DT: How is it possible without EM pictures from the purification, to know whether these particles are viral and appertain to a retrovirus, moreover a specific retrovirus?/

LM: Well, there were the pictures of the budding. We published images of budding which are characteristic of retroviruses. Having said that, on the morphology alone one could not say it was truly a retrovirus. For example, a French specialist of EMs of retroviruses publicly attacked me saying: "This is not a retrovirus, it is an arenavirus". Because there are other families of virus which bud and have spikes on the surface, etc. (4)

/DT: Why this confusion? The EM pictures did not show clearly a retrovirus?/

LM: At this period the best known retroviruses were those of type C, which were very typical. This retrovirus wasn't a type C and lentiviruses were little known. I myself recognised it by looking at pictures of Equine infectious anaemia virus at the library, and later of the visna virus. But I repeat, it was not only the morphology and the budding, there was RT...it was the assemblage of these properties which made me say it was a retrovirus. (5)

/DT: About the RT, it is detected in the culture. Then there is purification where one finds retroviral particles. But at this density there are a lot of others elements, among others those which one calls "virus-like"./

LM: Exactly, exactly. If you like, it is not one property but the assemblage of the properties which made us say it was a retrovirus of the family of lentiviruses. Taken in isolation, each of the properties isn't truly specific. It is the assemblage of them. So we had: the density, RT, pictures of budding and the analogy with the visna virus. Those are the four characteristics. (6)

/DT: But how do all these elements allow proof that it is a new retrovirus? Some of these elements could appertain to other things, "virus-like"...?/

LM: Yes, and what's more we have endogenous retroviruses which sometimes express particles - but of endogenous origin, and which therefore don't have pathological roles, in any case not in AIDS. (7)

/DT: But then how can one make out the difference?/

LM: Because we could "pass on" the virus. We passed on the RT activity in new lymphocytes. H. We got a peak of replication. We kept track of the virus. It is the assembly of properties which made us say it was a retrovirus. And why new? The first question put to us by Nature was: "Is it not a laboratory contamination? Is it perhaps a mouse retrovirus or an animal retrovirus?". To that one could say no! Because we had shown that the patient had antibodies against a protein of his own virus. The assemblage has a perfect logic! But it is important to take it as an assemblage. If you take each property separately, they are not specific. It is the assemblage which gives the specificity. (8)

/DT: But at the density of retroviruses, did you observe particles which seemed to be retroviruses? A new retrovirus?/

LM: At the density of 1.15, 1.16, we had a peak of RT activity, which is the enzyme characteristic of retroviruses. (9)

/DT: But could that be something else?/

LM: No..in my opinion it was very clear. It could not be anything but a retrovirus in this way. Because the enzyme that F. Barre-Sinoussi characterised biochemically needed magnesium, a little like HTLV elsewhere. It required the matrix, the template, the primer also which was completely characteristic of an RT. That was not open for discussion. At Cold Spring Harbour in September 1983, Gallo asked me whether I was sure it was an RT. I knew it, F. Barre-Sinoussi had done all the controls for that. It was not merely a cellular polymerase, it was an RT. It worked only with RNA primers, it made DNA. That one was sure of. (10)

/DT: With the other retroviruses you have met in your career did you follow the same process and did you meet the same difficulties?/

LM: I would say that for HIV it is an easy process. Compared with the obstacles one finds for the others...because the virus does not emerge, or indeed because isolation is sporadic - you manage it one time in five. I am talking about current research into others illnesses. One can cite the virus of Multiple Sclerosis of Prof. Peron. He showed me his work a decade ago and it took him around ten years to finally find a gene sequence which is very close to an endogenous virus. You see...it is very difficult. Because he could not "pass on" the virus, he could not make it emerge in culture. Whereas HIV emerges like couch grass. The LAI strain for example emerges like couchgrass. That's why it contaminated the others. (11)

/DT: With what did you culture the lymphocytes of your patient? With the H9 cell line?/

LM: No, because it didn't work at all with the H9. We used a lot of

cell lines and the only one which could produce it was the Tambon Iymphocytes. (12)

/DT: But using these kinds of elements it is possible to introduce other things capable of inducing an RT and proteins, etc.. /

LM: Agreed completely. That's why finally we were not very ardent about using immortal cell lines. To cultivate the virus en masse - OK. But not to characterise it, because we knew we were going to bring in other things. There are MT cell lines which have been found by the Japanese (MT2, MT4) which replicate HIV very well and which at the same time are transformed by HTLV. So, you have a mix of HIV and HTLV. It is a real soup. (13)

/DT: What's more it's not impossible that patients may be infected by other infectious agents? /

LM: There could be mycoplasmas...there could be a stack of things. But fortunately we had the negative experience with viruses associated with cancers and that helped us, because we had encountered all these problems. For example, one day I had a very fine peak of RT, which F. Barre-Sinoussi gave me, with a density a little bit higher, 1.19. And I checked! It was a mycoplasma, not a retrovirus. (14)

/DT: With the material purified at the retrovirus density, how is it possible to make out the difference between what is viral and what is not? Because at this density there's a stack of other things, including "virus-like" particles, cellular fragments.../

LM: Yes, that's why it is easier with the cell culture because one sees the phases of virus production. You have the budding. Charles Dauget (an EM specialist) looked rather at the cells. Of course he looked at the plasma, the concentrate, etc...he saw nothing major. Because if you make a concentrate it's necessary to make thinly sliced section [to see a virus with the EM], and to make a thin section it is necessary to have a concentrate at least the size of the head of a pin. So enormous amounts of virus are necessary. By contrast, you make a thin section of cells very easily and it's in these thin sections that Charles Dauget found the retrovirus, with different phases of budding. (15)

/DT: When one looks at the published electron microscope photographs, for you as a retrovirologist it is clear it's a retrovirus, a new retrovirus? /

LM: No, at that point one cannot say. With the first budding pictures it could be a type C virus. One cannot distinguish. (16)

/DT: Could it be anything else than a retrovirus? /

LM: No.. well, after all, yes .. it could be another budding virus. But there's a ... we have an atlas. One knows a little bit from familiarity, what is a retrovirus and what is not. With the morphology one can distinguish but it takes a certain familiarity. (17)

/DT: Why no purification?/

LM: I repeat we did not purify. We purified to characterise the

density of the RT, which was soundly that of a retrovirus. But we didn't take the peak...or it didn't work...because if you purify, you damage. So for infectious particles it is better to not touch them too much. So you take simply the supernatant from the culture of lymphocytes which have produced the virus and you put it in a small quantity on some new cultures of lymphocytes. And it follows, you pass on the retrovirus serially and you always get the same characteristics and you increase the production each time you pass it on. (18)

/DT: So the stage of purification is not necessary?/

LM: No, no, it's not necessary. What is essential is to pass on the virus. The problem Peron had with the multiple sclerosis virus was that he could not pass on the virus from one culture to another. That is the problem. He managed it a very little, not enough to characterise it. And these days to characterise means above all at the molecular standard. If you will, the procedure goes more quickly. So to do it : a DNA, clone this DNA, amplify it, sequence it, etc..So you have the DNA, the sequence of the DNA which tells you if it is truly a retrovirus. One knows the familiar structure of retroviruses, all the retroviruses have a familiar genomic structure with such and such a gene which is characteristic. (19)

/DT: So, for isolation of retroviruses the stage of purification is not obligatory? One can isolate retroviruses without purifying? /

LM: Yes .. one is not obliged to transmit pure material. It would be better, but there is the problem that one damages it and diminishes the infectivity of the retrovirus. (20)

/DT: Without going through this stage of purification, isn't there a risk of confusion over the proteins that one identifies and also over the RT which could come from something else?/

LM: No .. after all, I repeat if we have a peak of RT at the density of 1.15, 1.16, there are 999 chances out of 1,000 that it is a retrovirus. But it could be a retrovirus of different origin. I repeat, there are some endogenous retroviruses, pseudo-particles which can be emitted by cells, but even so, from the part of the genome that provides retroviruses. And which one acquires through heredity, in the cells for a very long time. But finally I think for the proof - because things evolve like molecular biology permitting even easier characterisation these days - it's necessary to move on very quickly to cloning. And that was done very quickly, as well by Gallo as by ourselves. Cloning and sequencing, and there one has the complete characterisation. But I repeat, the first characterisation is the belonging to the lentivirus family, the density, the budding, etc.. the biological properties, the association with the T4 cells. All these things are part of the characterisation, and it was us who did it. (21)

/DT: But there comes a point when one must do the characterisation of the virus. This means: what are the proteins of which it's composed? /

LM: That's it. So then, analysis of the proteins of the virus demands mass production and purification. It is necessary to do that. And there I should say that that partially failed. J.C. Chermann was in charge of that, at least for the internal proteins.

And he had difficulties producing the virus and it didn't work. But this was one possible way, the other way was to have the nucleic acid, cloning, etc. It's this way which worked very quickly. The other way didn't work because we had at that time a system of production which wasn't robust enough. One had not enough particles produced to purify and characterise the viral proteins. It couldn't be done. One couldn't produce a lot of virus at that time because this virus didn't emerge in the immortal cell line. We could do it with the LAI virus, but at that time we did not know that. (22)

/DT: Gallo did it? /

LM: Gallo? .. I don't know if he really purified. I don't believe so. I believe he launched very quickly into the molecular part, that's to say cloning . What he did do is the Western Blot. We used the RIPA technique, so what they did that was new was they showed some proteins which one had not seen well with the other technique. Here is another aspect of characterising the virus. You cannot purify it but if you know somebody who has antibodies against the proteins of the virus, you can purify the antibody/antigen complex. That's what one did. And thus one had a visible band, radioactively labelled, which one called protein 25, p25. And Gallo saw others. There was the p25 which he called p24, there was p41 which we saw... (23)

/DT: About the antibodies, numerous studies have shown that these antibodies react with other proteins or elements which are not part of HIV. And that they can not be sufficient to characterise the proteins of HIV. /

LM: No! Because we had controls. We had people who didn't have AIDS and had no antibodies against these proteins. And the techniques we used were techniques I had refined myself some years previously, to detect the src gene. You see the src gene was detected by immunoprecipitation too. It was the p60 [protein 60]. I was very dexterous, and my technician also, with the RIPA technique. If one gets a specific reaction, it's specific. (24)

/DT: But we know AIDS patients are infected with a multitude of other infectious agents which are susceptible to ... /

LM: Ah yes, but antibodies are very specific. They know how to distinguish one molecule in one million. There is a very great affinity. When antibodies have sufficient affinity, you fish out something really very specific. With monoclonal antibodies you fish out really ONE protein. All of that is used for diagnostic antigen detection. (25)

/DT: For you the p41 was not of viral origin and so didn't belong to HIV. For Gallo it was the most specific protein of the HIV. Why this contradiction? /

LM: We were both reasonably right. That's to say that I in my RIPA technique...in effect there are cellular proteins that one meets everywhere - there's a non-specific "background noise", and amongst these proteins one is very abundant in cells, which is actin. And this protein has a molecular weight 43000kd. So, it was there. So I was reasonably right, but what Gallo saw on the other hand was the gp41 of HIV, because he was using the Western Blot. And that I have recognised. (26)

/DT: For you p24 was the most specific protein of HIV, for Gallo not at all. One recognises thanks to other studies that the antibodies directed against p24 were often found in patients who were not infected with HIV, and even in certain animals. In fact today, an antibody reaction with p24 is considered non specific. /

LM: It is not sufficient for diagnosing HIV infection. (27)

/DT: No protein is sufficient? /

LM: No protein is sufficient anyway. But at the time the problem didn't reveal itself like that. The problem was to know whether it was an HTLV or not. The only human retrovirus known was HTLV. And we showed clearly that it was not an HTLV, that Gallo's monoclonal antibodies against the p24 of HTLV did not recognise the p25 of HIV. (28)

/DT: At the density of retroviruses, 1.16, there are a lot of particles, but only 20% of them appertain to HIV. Why are 80% of the proteins not viral and the others are? How can one make out the difference?/

LM: There are two explanations. For the one part, at this density you have what one calls microvesicles of cellular origin, which have approximately the same size as the virus, and then the virus itself, in budding, brings cellular proteins. So effectively these proteins are not viral, they are cellular in origin. So, how to make out the difference?! Frankly with this technique one can't do it precisely . What we can do is to purify the virus to the maximum with successive gradients, and you always stumble on the same proteins. (29)

/DT: The others disappear?/

LM: Let's say the others reduce a little bit. You take off the microvesicles, but each time you lose a lot of virus, so it's necessary to have a lot of virus to start off in order to keep a little bit when you arrive at the end. And then again it's the molecular analysis, it's the sequence of these proteins which is going allow one to say whether they are of viral origin or not. That's what we began for p25, that failed ...and the other technique is to do the cloning, and so then you have the DNA and from the DNA you get the proteins. You deduce the sequence of the proteins and their size and, you stumble again on what you've already observed with immunoprecipitation or with gel electrophoresis. And one knows by analogy with the sizes of the proteins of other retroviruses, one can deduce quite closely these proteins. So you have the p25 which was close to the p24 of HTLV, you have the p18..in the end you have the others. On the other hand the one which was very different was the very large protein, p120. (30)

/DT: Today, are the problems about mass production of the virus, purification, EM pictures at 1.16, resolved? /

LM: Yes, of course. (31)

/DT: Do EM pictures of HIV from the purification exist?/

LM: Yes. of course. (32)

/DT: Have they been published? /

LM: I couldn't tell you...we have some somewhere .. but it is not of interest, not of any interest. (33)

/DT: Today, with mass production of the virus, is it possible to see an EM, after purification, of a large number of viruses?/

LM: Yes, yes. Absolutely. One can see them, one even sees visible bands. (34)

/DT: So for you HIV exists?/

LM: Oh, it is clear. I have seen it and I have encountered it. (35) *

/Notes: Go here <http://www.virusmyth.com/aids/hiv/epreplyintervlm.htm> for the reply by the Perth Group./

VIRUSMYTH HOMEPAGE <http://www.virusmyth.com/aids/index.htm>

Nov 26th 2020,
To: Editorial Board Eurosurveillance
European Centre for Disease Prevention and Control (ECDC)
Gustav III:s Boulevard 40
16973 Solna
Sweden

Subject: External Review and request to retract the paper of Corman et al, published in Eurosurveillance January 23, 2020.

Dear editorial board Eurosurveillance,

We, an international consortium of life-science scientists, write this letter in response to the article “Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR” published in Eurosurveillance (January 23rd, 2020) and co-authored by Victor M Corman , Olfert Landt , Marco Kaiser , Richard Molenkamp, Adam Meijer, Daniel KW Chu, Tobias Bleicker , Sebastian Brünink, Julia Schneider , Marie Luisa Schmidt , Daphne GJC Mulders , Bart L Haagmans , Bas van der Veer , Sharon van den Brink, Lisa Wijsman, Gabriel Goderski, Jean-Louis Romette, Joanna Ellis, Maria Zambon, Malik Peiris, Herman Goossens, Chantal Reusken, Marion PG Koopmans, and Christian Drosten.

This paper (hereafter referred to as “Corman-Drosten paper”), published by “Eurosurveillance” on 23 January 2020, describes an RT-PCR method to detect the novel Corona virus (also known as SARS-CoV2). After careful consideration, our international consortium of Life Science scientists found the Corman-Drosten paper is severely flawed with respect to its biomolecular and methodological design. A detailed scientific argumentations can be found in our review “External peer review of the RTPCR test to detect SARS-CoV2 reveals 10 major scientific flaws at the molecular and methodological level: consequences for false positive results”, which we herewith submit for publication in Eurosurveillance.

Further, the submission date and acceptance date of this paper are January 21st and January 22nd, respectively. Considering the severe errors in design and methodology of the RT-PCR test published by “Eurosurveillance”, this raises the concern whether the paper was subjected to peer-review at all.

A previous request from our side (Dr. P. Borger; email 26/10/2020) to the editors of “Eurosurveillance” to provide the peer review report of the Corman-Drosten paper has not been complied with. We have enclosed your email reply (dated 18/11/2020) indicating that you do not wish to disclose important information to solve this conundrum.

We are confident that you will take our scientific objections seriously and recognize that there is no alternative but to accept our request to retract the Corman-Drosten paper.

Sincerely,

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

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

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

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

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

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

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

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ABSTRACT

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

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

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

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

CONCISE REVIEW REPORT

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

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

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

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

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

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

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

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

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

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

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

1. The primers and probes:

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

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

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

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

2. The temperature at which all reactions take place:

a) DNA melting temperature (>92°)

b) DNA amplification temperature (TaqPol specific)

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

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

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

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

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

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

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

MINOR CONCERNS WITH THE CORMAN-DROSTEN PAPER

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

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

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

MAJOR CONCERNS WITH THE CORMAN-DROSTEN PAPER

A) BACKGROUND

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

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

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

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

B) METHODS AND RESULTS

1. Primer & Probe Design

1a) Erroneous primer concentrations

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

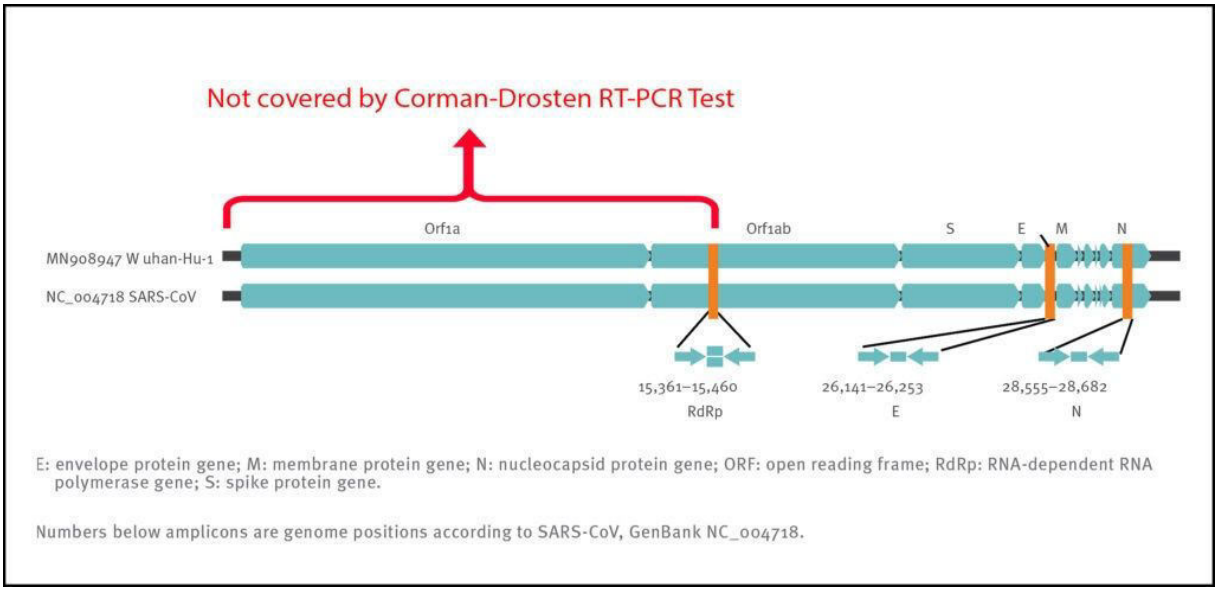
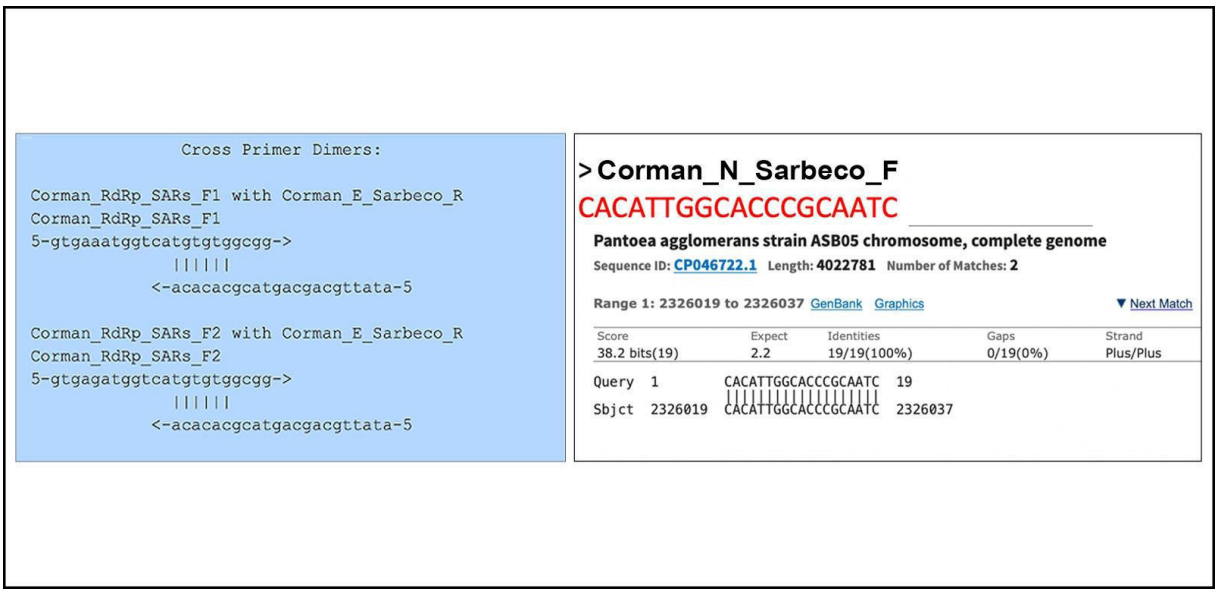


Figure 3: A test with Thermofischer's primer dimer web tool reveals that the RdRp forward primer has a 6bp 3`prime homology with Sarbeco E Reverse (left box). Another test reveals that there is a perfect match for one of the N-primers to a clinical pathogen (Pantoea) found in immuno-compromised patients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



2. Reaction temperature

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

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

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

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

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

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

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

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

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

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

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

GC% 59,09 — TM 63,74°

Difference of almost 10°

GC% 28,00 — TM 53,56°

GC% 34,62 — TM 58,29°

GC% 45,45% — TM 60,93°

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

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

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

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

3. The number of amplification cycles

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

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

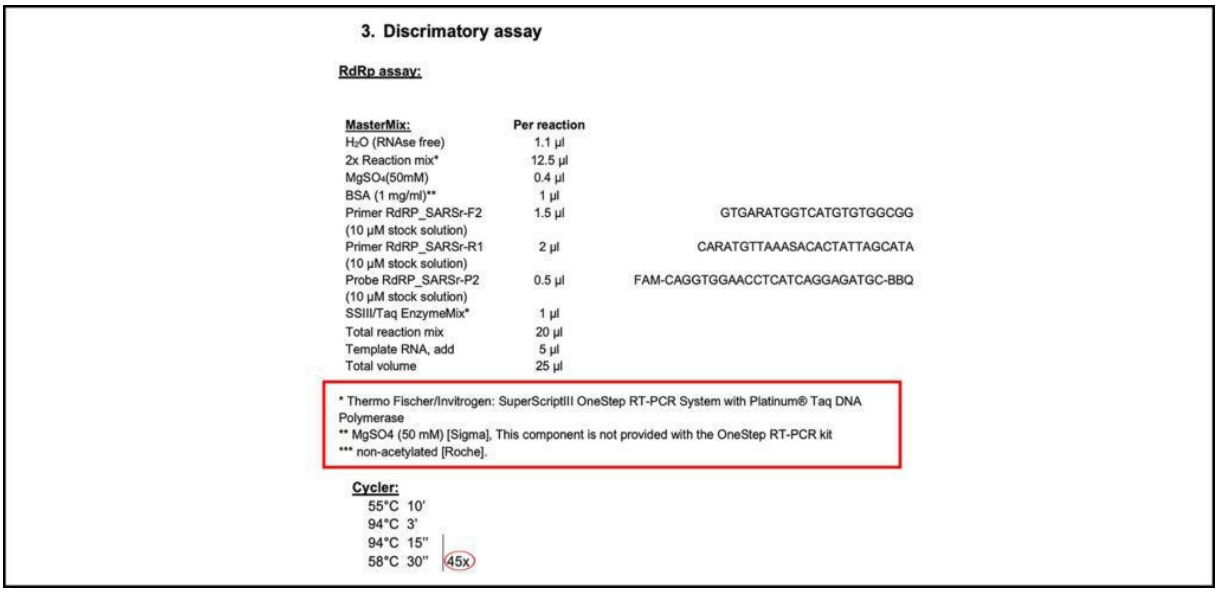
Citing Jaafar et al. 2020 [3]:

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

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

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

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



3. Discrimatory assay

RdRp assay:

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

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

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

4. Biomolecular validations

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

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

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

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

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

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

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

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

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

Third, the Corman-Drosten paper states:

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

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

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

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

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

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

6. Standard Operational Procedure (SOP) is not available

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

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

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

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

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

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

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

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

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

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

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

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

9. Authors as the editors

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

SUMMARY CATALOGUE OF ERRORS FOUND IN THE PAPER

The Corman-Drosten paper contains the following specific errors:

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

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

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

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

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

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

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

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

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

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

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

CONCLUSION

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

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

REFERENCES

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

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

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

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

Archive: https://archive.is/0qRmZ

[5] Google Analytics - COVID19-deaths worldwide: https://bit.ly/3fndemJ

Archive: https://archive.is/PpqEE

[6] Laboratory testing for COVID-19 Emergency Response Technical Centre, NIVD under

China CDC March 15th, 2020: http://www.chinacdc.cn/en/COVID19/202003/P020200323390321297894.pdf

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

handbook.pdf

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

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

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

[10] Wolfel et al., Virological assessment of hospitalized patients with COVID-2019

https://www.nature.com/articles/s41586-020-2196-x

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

Suplementary material by Kevin Mckernan, Corman-Drosten Primer Dimer Results with Thermofischer Primer Dimer Web Tool

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

[13] Marra MA, Steven JMJ, Caroline RA, Robert AH, Angela BW et al. (2003) Science. The

Genome sequence of the SARS-associated coronavirus. Science 300(5624): 1399-1404.

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

[15] Borger P. A SARS-like Coronavirus was expected but nothing was done to be prepared. Am J Biomed Sci Res 2020. https://biomedgrid.com/pdf/AJBSR.MS.ID.001312.pdf

https://www.researchgate.net/publication/341120750_A_SARS-like_Coronavirus_was_Expected_but_nothing_was_done_to_be_Prepared;

Archive: https://archive.is/i76Hu

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

[17] Official recommendation of the Corman-Drosten protocol & manuscript by the WHO,published on January 13th 2020 as version 1.0 of the document:

https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287a1b17c111902.pdf; archive: https://bit.ly/3m3jXVH

[18] Official WHO-recommendation for the Corman / Drosten RT-qPCR-protocol, which directly derives from the Eurosurveillance-publication, document-version 2-1, published on

17th January 2020:
https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2

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

Archive: https://bit.ly/2TqXBjX

[20] Instructions For Use LightMix SarbecoV E-gene plus EAV Control, TIB-Molbiol & Roche Molecular Solutions, January 11th 2020:
https://www.roche-as.es/lm_pdf/MDx_40-0776_96_Sarbeco-E-gene_V200204_09164154001 (1).pdf

Archive, timestamp - January 11th 2020: https://archive.is/Vulo5;

Archive: https://bit.ly/3fm9bXH

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

Archive: https://archive.is/CDEUG

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

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

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

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

Additional literature:

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

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

Addendum

Background:

After submitting our review report on Corman *et al.* (referred hereinafter as CD-report) and republishing it on a scientific preprint server [50] and Researchgate.net [51] we offered the report for public discussion at cormandrostenreview.com on 27th November 2020. The scientific community provided additional literature, references, and analyses concerning the CD-report and the Corman *et al*. manuscript. Several "advocatus diaboli" confronted us with correct or assumed problems in our report. The most common critique of the CD-report was the lack of "wet lab" experiments to support our concerns over the technical flaws in the PCR protocol.

Aim:

This vibrant debate on our CD report has provided additional information worthy of further public documentation to address these critiques. We summarize the current published knowledge of "wet lab testing", routine diagnostic use and validation of the original PCR-Protocol described by Corman *et al.* Further, this addendum highlights that independent research groups (some of them with Corman and/or Drosten as author) also pointed out important concerns with the original manuscript and Corman PCR protocol distributed by the WHO. Many of these references were already provided by the authors of the original CD-report but it is worth underscoring their relevance to the formation of our critiques of the CD manuscript.

Methods:

We searched the literature for 'SARS-CoV-2 qPCR' and 'Corman' or 'Charité'. Then we combined these references with those provided by other scientists working in relevant Life Sciences/data analysis fields.

In the first section of the addendum, the publications will be discussed point by point, highlighting their findings in relation to the CD-report. In a second section, additional aspects about the Corman *et al*. publication are discussed. This spans a meta-analysis of the unusual peer-review process, timeframes, and further technical vulnerabilities of the Corman *et al*. PCR-protocol.

An additional concern was raised about the CD-report regarding the discussion of appropriate controls. We cite several studies that underscore the importance of internal controls in assessing viral load and the lack of such internal controls in the Corman qPCR method. These internal controls are required for normalizing swab sampling variance and

they are critical for interpreting viral load. They are notably absent from the Corman PCR protocol. Several people also expressed confusion regarding the NCBI submissions provided by Corman *et al*. The sequences provided lack two of the target gene sequences Corman *et al.* claim to target. The only sequences referenced in the manuscript are listed (KC633203, KC633204, KC633201, GU190221, GU190222, GU190223) and none of these have sequences that match their N and E gene primers. This not only brings their validation into question but also prevents others from reproducing the work presented in Corman *et al.*

Results:

We present 20 scientific publications providing 'wet lab' evidence of the performance of the Corman *et al.* PCR protocol. Of those, 17 found problems with incorrect primer design (mismatches, dimer formation, melting temperature) in the SARS-CoV-2 specific "confirmatory" test named RdRp-PCR for "RNA-dependent RNA-polymerase" or the E-gene assay.

These documented problems include:

- Documented primer dimers and False Positives in non-template controls (NTCs)
- Documented poor sensitivity and False Negatives compared to other assays
- No internal control to normalize the sample preparation variability and its impact on viral load estimation
- No defined Ct for calling samples "Positive cases"
- Poorly documented positive controls and sequences used in their study

Conclusion:

We believe the references provided in this addendum itemize the scientific consensus evident in the literature regarding the flaws in the original PCR detection method for SARs-CoV-2 published by Corman *et al.*. Further, since several important flaws were published in peer-reviewed journals, the lack of correction of the original PCR protocol by either Eurosurveillance or as an update in the Charité-WHO protocol brings into question the scientific integrity of the authors of Corman *et al.* These references settle any remaining debate that the Corman *et al.* manuscript should be retracted on technical grounds alone. The rapidity of the peer-review and conflicts of interest are even more troubling.

Addendum: Peer reviewed literature and preprints covering wet experiments, *in silico* analysis of the Corman Drosten protocol-design, meta-data analysis on EuroSurveillance.org and further discussion

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Index

Note: sentences written in italics are original citations from the respective publications

Section 1:

A. Wet lab evidence of primer design flaws

The primer pair for the RdRp gene was shown to create a positive PCR test result in the absence of SARS-CoV-2. This can happen when the primer design is suboptimal and the primers react with themselves in the absence of the virus. Insufficient test specificity and primer design flaws seen in Corman-Drosten's SARS-CoV2 qPCR assay creates a high number of false positive and false negative results.

a. Background and Pinollel et al. (Letter to the editor of Eurosurveillance)

We have listed 20 references that give compelling wet-lab evidence for flaws in primer design and methodological validation of the PCR testing protocol by Corman *et al*. These studies nullify the most common complaint voiced (no wet-lab evidence) regarding the retraction letter.

There is no need for the authors of the Corman-Drosten (CD manuscript) retraction request to perform wet-lab experiments to prove these deficiencies as those experiments are already evident in fully peer-reviewed articles. These papers represent diverse labs with diverse authors and different jurisdictional influences on the scientific funding and research.

Initially, it is important to underscore the other complaint already evident with the CD manuscript.

Pillonel et al. - Letter to the editor: SARS-CoV-2 detection by real-time RT-PCR [16]:

"After careful review of the initial manuscript and analysis of SARS-CoV-2 and other coronavirus sequences, it appeared that the proposed RdRp reverse primer contained an incorrect degenerate base (S), that does not match with the SARS-CoV-2 RNA sequence, as shown in the alignment of Corman et al. Figure 2."
[...]
"These observations based on in silico alignments should be confirmed by wet-laboratory experiments, but they could explain the lower sensitivity of the RdRp RT-PCR also shown by Vogels et al. and point towards potential improvements."

"As the pandemic spreads, many laboratories worldwide, including in low-resource countries that may not rely on expensive commercial kits, implement routine

diagnostic tests. Thus, we think that such information is critical to ensure a proper detection of SARS-CoV-2 infections, allowing efficient isolation and preventing further transmission of the virus."

Corman et. al: Authors' response: SARS-CoV-2 detection by real-time RT-PCR [31]:

"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."

Since Nalla *et al.* is cited in this author's response as reference, also see section ***16. Nalla et al.*** in this Addendum.

This Addendum challenges the authors' response (Corman *et al*.) and claims to Pillonel *et al*.'s letter to the editor (Table 1).

Addendum - Corman Drosten Review Report by an International Consortium of Scientists in Life Sciences (ICSLS): Peer reviewed literature and preprints covering wet-lab experiments, *in silico* analysis of Corman Drosten protocol-design, meta-data analysis on EuroSurveillance.org and further discussion

Last Updated: 12.01.2021

Table 1: Main findings in the publications reviewed

Publication	Proof of false positive (FP) or low sensitivity (LS)	Discussion of high CT	Detected mismatches	Primer dimers	Authors modified primers	Reason
Muenchhoff et al	RdRp (LS)	E-gene (≥37) RdRp gene (≥40)	In RdRp reverse		RdRp reverse	high difference in melting temperature
Jung et al	RdRp (FP)					
Etievant et al	E-gene (FP) RdRp gene (LS)			Detected with primer contamination		
Gand et al	N-Gene (LS)		N-gene forward and reverse RdRp reverse RdRp probe			Mentions WHO needs to update Corman errors
Konrad et al	E-Gene (FP)	E-gene, FP ≥ 35		Discussed for E-gene		
Sethuraman et al						Only review
Nalla et al	N-gene (LS) RdRp-gene (LS)					
Vogels et al	RdRp-gene (LS)	For N gene of CDC only	RdRp-gene			
Kuchinski et al						
Ratcliff et al			RdRp			Correcting the mismatch
Jaeger et al				Dimer formation with Taqman or fluorogenic probes detected		CDC primers only
Khan et al			RdRp reverse (T)			In silico
Opota et al	E-Gene (FP+FN)					
Barra et al						Higher primer concentration in order to improve detection limit
Santos et al			RdRp reverse (T)			
Anantharajah et al	RdRp (LS)		RdRp reverse (T)			
Nalla et al	RdRp (LS) E gene (LS)					
Dahdouh et al		10-16 Ct variance in Sample prep. Requires human amplicon to normalize				Critical to have Internal controls
Poljak et al						Critical to have Internal controls
Boutin et al	15% disagreement					Critical to have Internal controls
Pfefferle et al					Modified primers to prevent primer dimers	

b. Review of the literature

1. Muenchhoff *et al.*

Muenchhoff *et al.* compare seven different labs using various PCR protocols including the primers described in the CD manuscript. Six out of seven laboratories in the Muenchhoff *et al*. paper [1] tested the original primer pairs described in the Corman-Drosten paper. Muenchhoff *et al*. also refers to the official WHO-recommendation of the protocol [2].

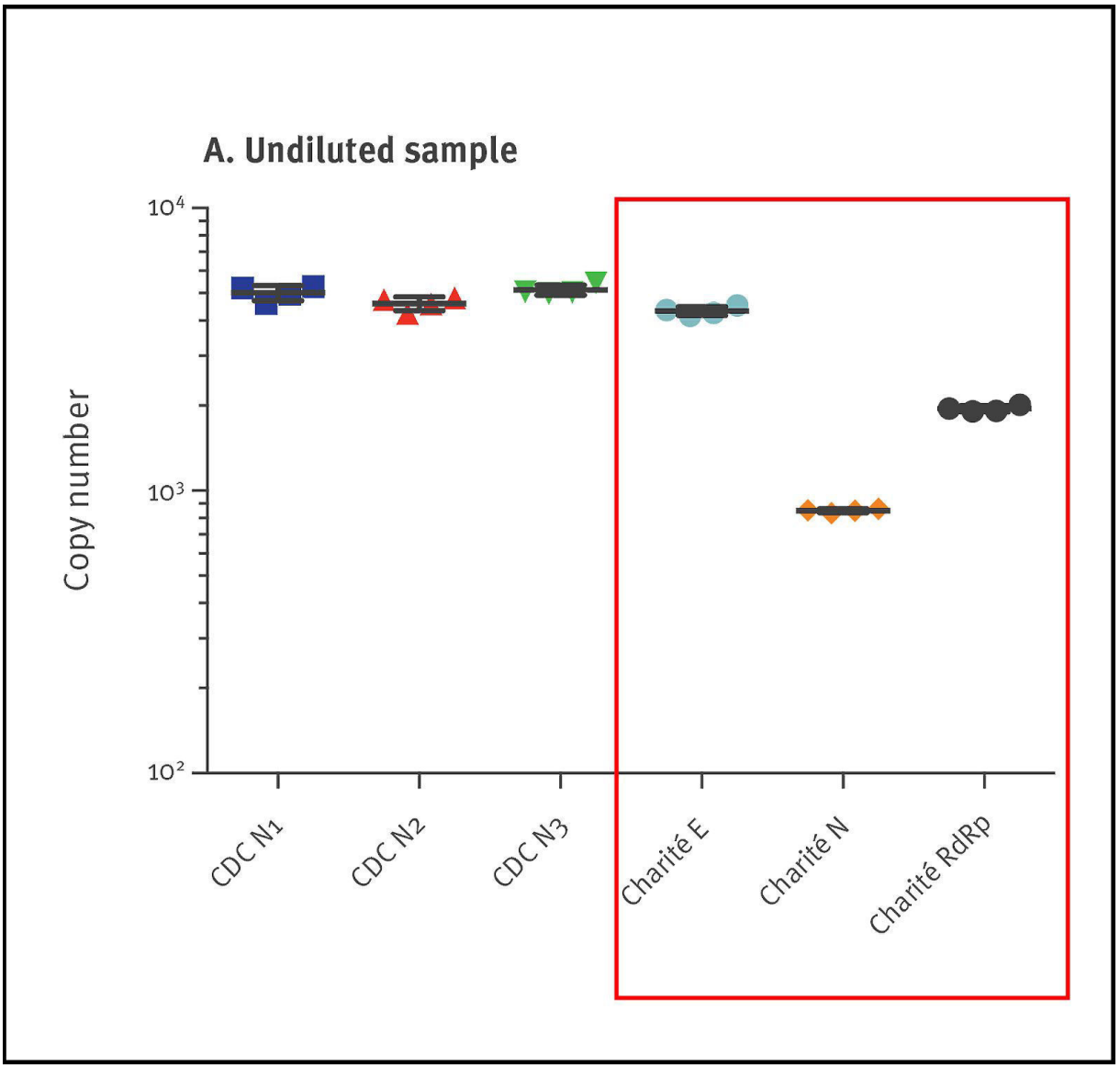
According to table 1 in the Muenchhoff *et al*. paper, the Corman-Drosten protocol components (primers, gene assays, etc.) are labeled and referred to as “Charité genes” and TIB-Molbiol is listed as the manufacturer of the corresponding primers/probes.

As a proficiency test for inter-laboratory performance evaluation, a series of 10-fold dilutions of one of the SARS-CoV-2 PCR positive RNA samples was sent out to all seven laboratories. As a result, 5 of 6 laboratories were able to find as low as 5 copies of SARS-CoV-2 RNA by Charité E-gene PCR, and all 50 or fewer copies by the Charité RdRp gene PCR. The three labs amplifying the Charité N-gene PCR managed to detect 5 of the spiked RNA molecules.

In parallel to the intra-laboratory testing of the RNA dilution series, the main authors of the manuscript compared the sensitivity of different primer pairs with a digital droplet PCR in their laboratory (Laboratory 1).

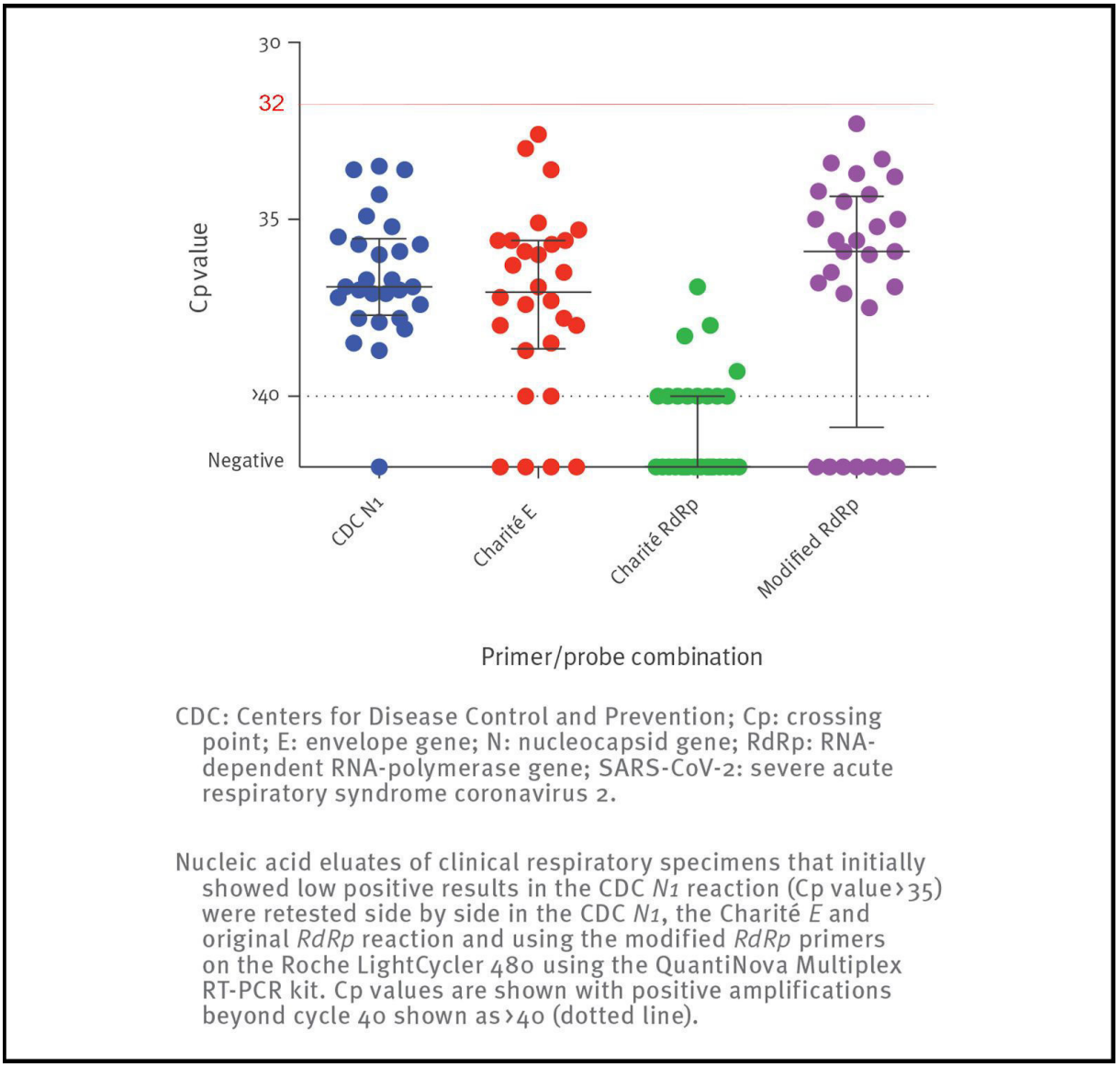
Based on the digital droplet PCR, the authors concluded that the “Charité E gene” primer pair performance is comparable with the “CDC N primer pairs”; both show similar sensitivity, but the N gene and the RdRp gene assays are **significantly less sensitive** with the positive RNA samples tested (Figure 1).

Figure 1, taken from Muenchhoff *et al.*: Digital droplet PCR quantification of the distributed dilution series of nucleic acid eluate of SARS-CoV-2-positive clinical material, Germany, March 2020.



In addition, a test of 28 samples derived from pre-tested CDC N1-gene positive patient-samples in Laboratory 1 revealed that all Charité primer pairs showed a Ct with a median of around 37 (CDC N1 and Charite' E) and 40 or higher (Charite' RdRp) and a "modified" improved Charite' RdRp showed a Ct of 36 as median (Figure 2). None of the patients' samples were positive at a Ct of 32 or lower.

Figure 2 taken from Muenchhoff *et al. (Figure 3)*:
RT-PCR results of respiratory samples with low positivity, SARS-CoV-2 detection, Germany, March 2020 (n = 28 samples). The Charité RdRp assay is the worst performing.



CDC: Centers for Disease Control and Prevention; Cp: crossing point; E: envelope gene; N: nucleocapsid gene; RdRp: RNA-dependent RNA-polymerase gene; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2.

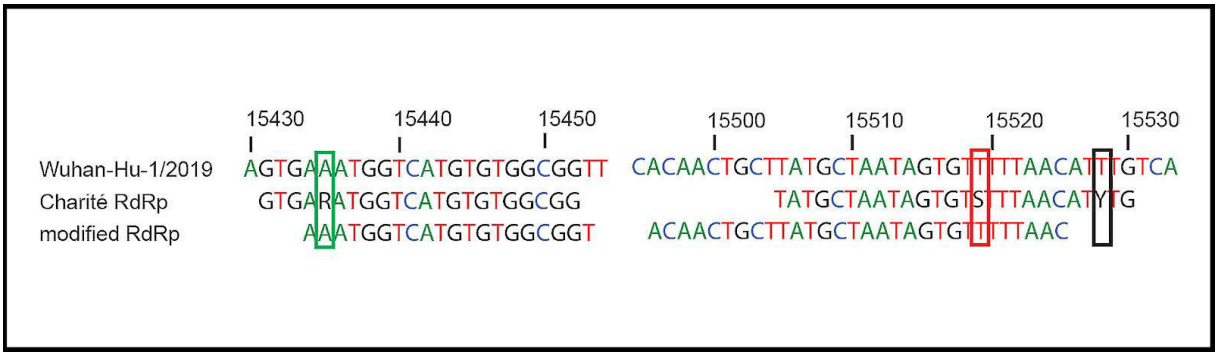
Nucleic acid eluates of clinical respiratory specimens that initially showed low positive results in the CDC *N1* reaction (Cp value›35) were retested side by side in the CDC *N1*, the Charité *E* and original *RdRp* reaction and using the modified *RdRp* primers on the Roche LightCycler 480 using the QuantiNova Multiplex RT-PCR kit. Cp values are shown with positive amplifications beyond cycle 40 shown as›40 (dotted line).

This modified reverse RdRp primer was created by the authors due to a mismatch of one of the bases in the original Charité primer to the reference sequence Wuhan-Hu-1/2019, which was replaced by the correct “T” and the selection of another “T” in a second position, where the original Charité primer had an ambiguity base (C or T) which should be a T. Further, the Muenchhoff *et al.* authors claimed that:

"Based on computation using Primer Express v3.0 (Applied Biosystems, Dreieich, Germany) annealing temperatures were predicted to be 64 °C for the RdRp forward and 51 °C for the RdRp reverse primer of the Charité protocol. This temperature difference may result in reduced PCR efficiency" [1]

Both primer sequences were shown in their supplemental figure S1 (note: the reverse primer is given as a complementary sequence). (Figure 3)

Figure 3 taken from Muenchhoff *et al*. (figure S1):
The forward primer and the reverse complement of the reverse primer of the RdRp reaction from the Charité protocol is aligned to the reference sequence Wuhan-Hu-1/2019 (NCBI NC_045512.2). The red box indicates an ambiguity base S, i.e. G or C, at a position where T should be the reverse complement. The black box indicates an ambiguity base Y, i.e. T or C, at a position where T would exist, and the green box indicates an R where A can be used based on currently available sequence data.



The modified RdRp primer pair now has the correct melting temperature, however the modified reverse primer is now unusually 30 bp long. (Table 1)

Table 1: Modified RdRp primer pair, Length, Tm, GC% - values - values according to Primer Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/)

RdRp	**Sequence (5' -> 3')**	Length	Tm	GC%	Self complementarity
Forward primer	AAATGGTCATGTGTGGCGGT	20	60.54	50.00	4.00
Reverse primer	GTTAAAAACACTATTAGCATAAGCAGTTGA	30	59.53	30.00	5.00

This need for primer modification is a direct result of the authors of the Corman *et al.* protocol skipping mandatory and simple-to-test primer design QC steps. Screening for primer dimers or hairpins is a crucial step to avoid false positive as well as false negative

results. Open-source software such as the web tool by Thermofisher [3] is freely available on the internet to perform this critical screening and is shown below this review of the Muenchhoff *et al*. section. (Figure 5)

Conclusion Muenchhoff *et al.*

The rapid communication-publication (also published in Eurosurveillance) concludes that the RdRp assay in the Corman-Drosten paper is deficient and needs to be replaced. The paper demonstrates sensitivity issues, which would support false negatives being generated by the test.

"A reduced sensitivity was noted for the original Charité RdRp gene confirmatory protocol, which may have impacted the confirmation of some COVID-19 cases in the early weeks of the pandemic. The protocol needs to be amended to improve the sensitivity of the RdRp reaction." [1]

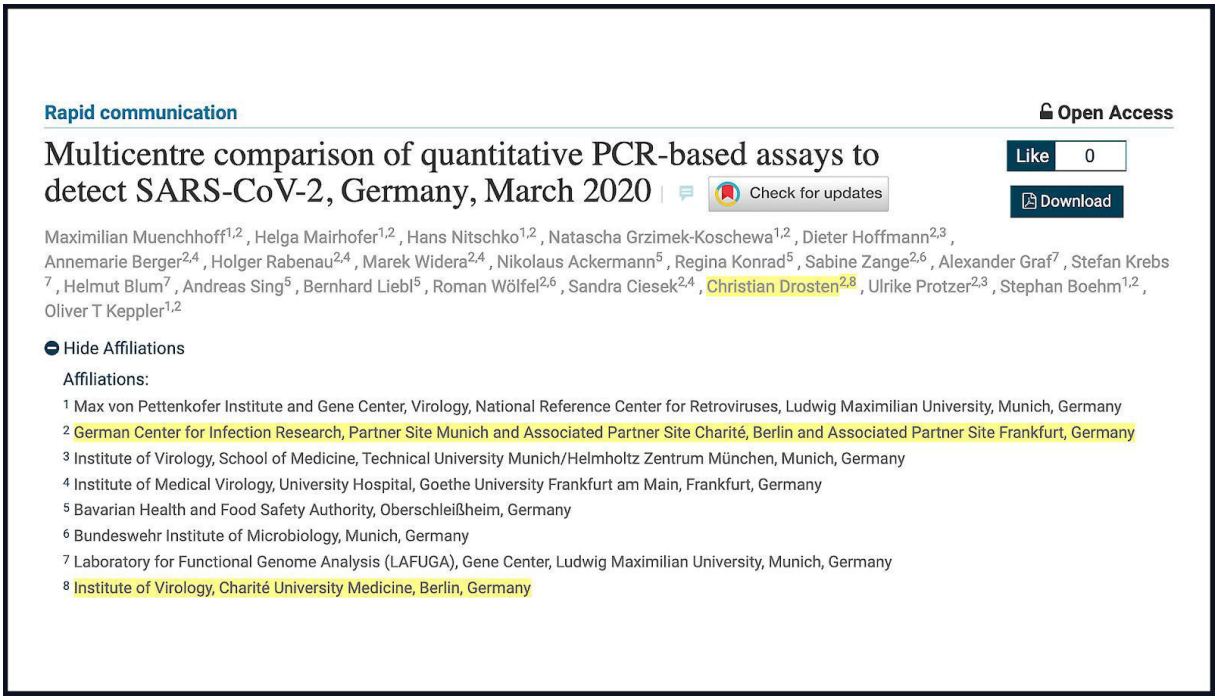
Further discussion of Muenchhoff *et al.*

1. The fact that the Corman *et al.* primers were given to testing companies (Labor Berlin, Tib Molbiol) and commercially sold as Light Mix diagnostic Test kits (LightMix® Modular SARS-CoV / COVID19, RdRp / LightMix®Modular SARS-CoV / COVID19, E-gene, TIB Molbiol, Roché diagnostics) and cemented into WHO guidelines prior to peer-review should concern everyone. This is 'science by press-release' where authoritative bodies (the WHO) are used to advertise a manuscript before it has seen proper peer-review. After the PCR protocol is pushed through the WHO, we additionally see a rushed 24 hour peer-review, while furthermore the authors being on the editorial board of the journal (Eurosurveillence) performing the review. This is a dangerous practice when undisclosed conflicts of interest (COIs) exist. It is now known to have produced erroneous results and contributed to global lockdowns.
2. The author's urgency in communication with the WHO, is not replicated in addressing the errors in Muenchhoff *et al.* which Drosten is an author of. These known errors were published on June 18th 2020 and yet the WHO primers are not updated as of today! Why the race to get these primers to testing companies and onto the WHO website in January 2020? Why the lack of urgency in addressing the false negatives (FNs) and false positives (FPs) 6 months after publishing Muenchhoff? Testing labs generate more revenue with higher positivity tests due to contact trace testing. This COI may explain the different urgency?
3. Christian Drosten is co-author of the Muenchoff *et al*. publication, which was released on 18th June 2020 at Eurosurveillance (Figure 4). The study clearly

concludes that the Corman-Drosten paper RdRp primer designs must be exchanged and/or removed from the protocol due to sensitivity issues. Other papers provided below highlight water samples (NTCs) amplifying. Thus, the protocol lacks sensitivity for the RNA target and specificity in the signal it provides. It produces both FPs and FNs.

4. In the Muenchhoff *et al.* publication Christian Drosten does not properly disclose his COIs and affiliations (Figure 4). As in the Corman-Drosten paper, his affiliation as Director of Virology at Labor Berlin is not listed, a laboratory which operates commercially within the PCR-testing realm. [5]

Figure 4: Christian Drosten fails to list his affiliations properly: He is Director of Virology at Labor Berlin, a commercially oriented company which offers PCR-testing.



Rapid communication

Open Access

Multicentre comparison of quantitative PCR-based assays to detect SARS-CoV-2, Germany, March 2020

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Hide Affiliations

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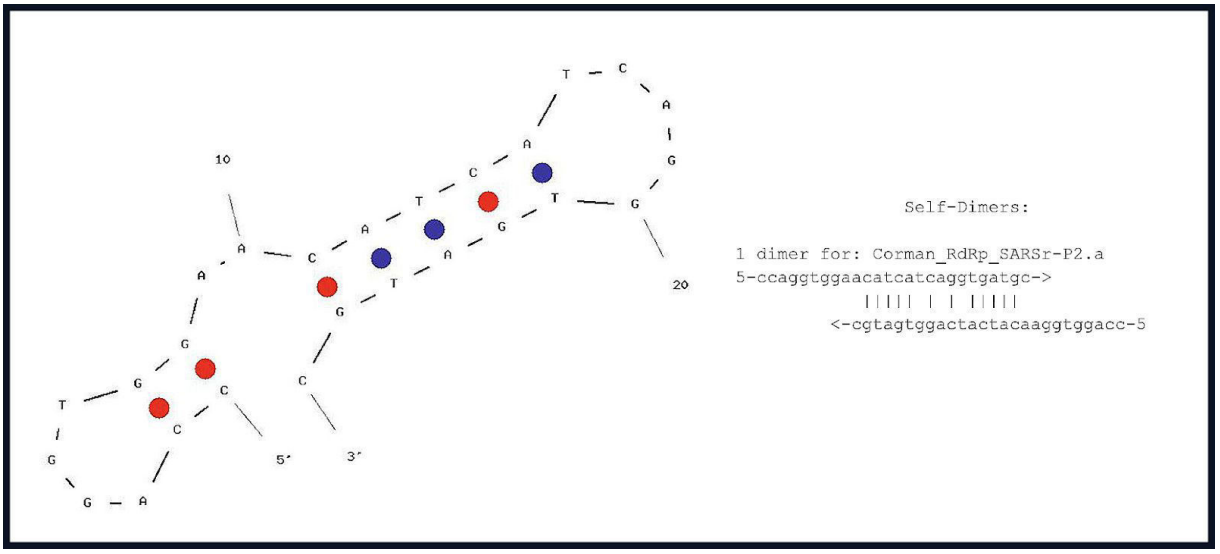
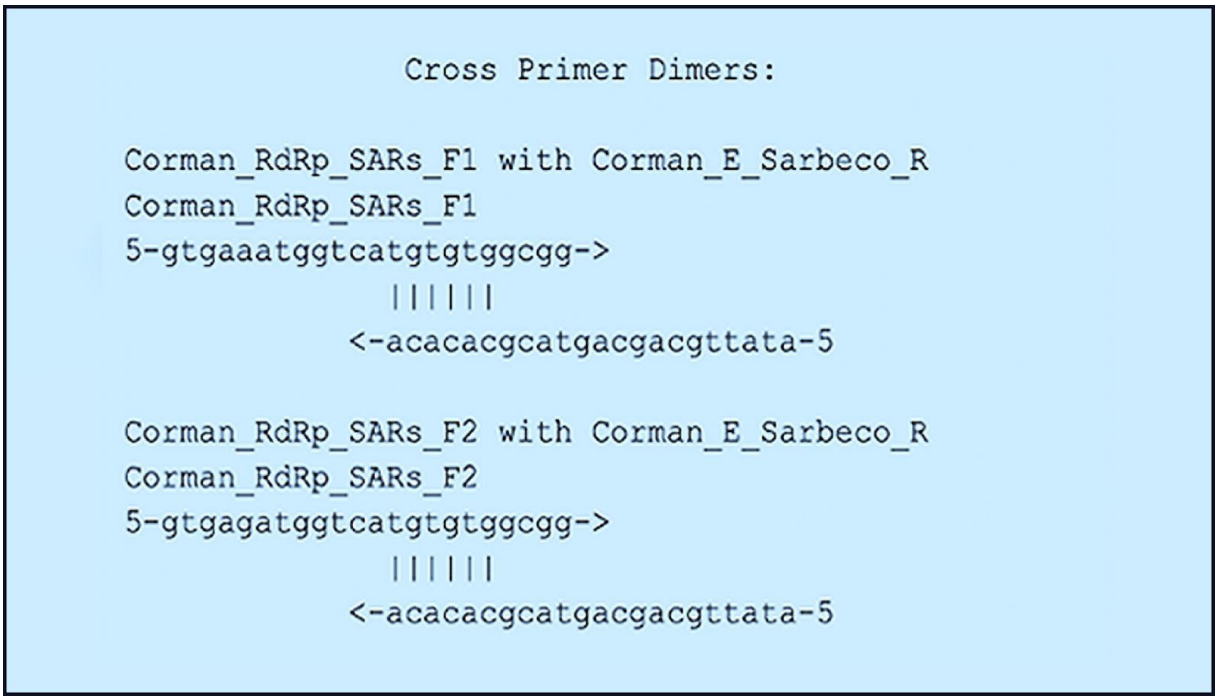
[8] Institute of Virology, Charité University Medicine, Berlin, Germany

Figure 5: Corman-Drosten protocol RdRp probe, hairpins and homodimers.



RdRp gene primers also have a homology to the E-gene primers, which was already discussed in the main review report [4], see Figure 6.

Figure 6: A test with Thermo Fisher's primer dimer web tool reveals that the RdRp forward primer has a 6bp 3`prime homology with Sarbeco E Reverse.



While most labs run these tests in different wells (1-plex), it is certainly risky practice to have primer dimers between 1-plexes, especially when factoring in that liquid handling of millions of tests can create numerous contaminations. Such primer contaminations are not just a theoretical risk but are in fact reported in the peer-reviewed literature referred to below.

2. Jung *et al.*

The authors tested several PCR primer pairs for amplification of isolated N from SARS-CoV-2 infected cell cultures. As a result Jung *et al*. did not recommend the Corman *et al.* RdRp PCR (named Charite PCR in the publication) for diagnostic purposes.

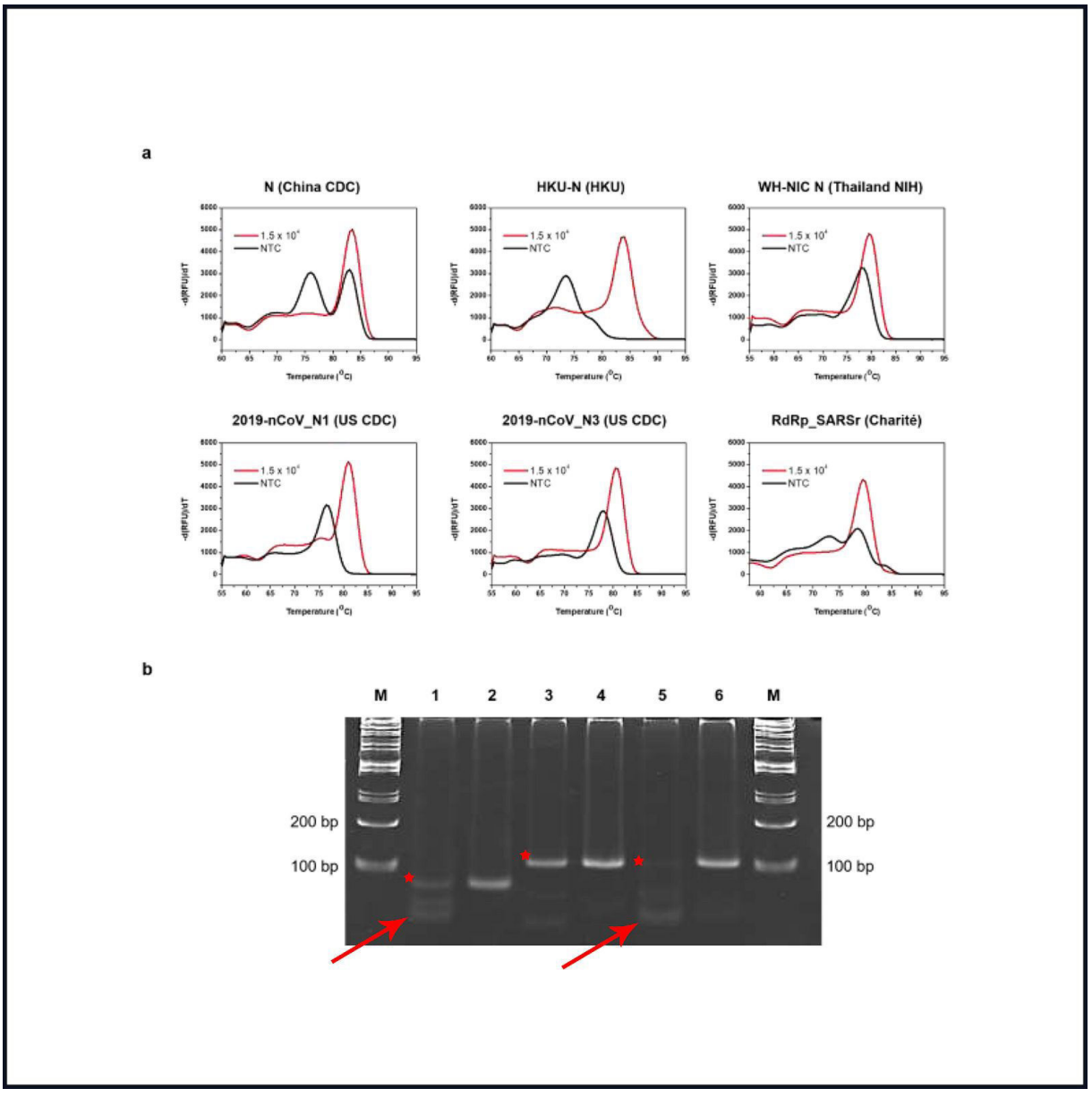
Jung *et al*. clearly refute a commonly voiced misconception, that reduced sensitivity of the Corman *et al.* protocol could only manifest itself with false negatives and should not create false positives.

"Unexpected amplifications from NTC samples were observed with the RdRp_SARSr (Charité) set. The electrophoresis and melting curve analysis showed non-specific amplification at lower positions (Lane 5, Figure S5b) and temperatures (Figure S5a)." [7] (Figure 7)

Jung *et al.* further demonstrate these primers have reduced sensitivity as reported by Muenchhoff *et al.* False negatives and false positives are generated with the Corman-Drosten primer design.

Promiscuous primers not only fail to amplify targets in some samples, they also amplify non-specific sequences in other samples which they should not amplify. **In this case they amplify water** (NTC). The authors demonstrate the Charité RdRp PCR generate positive water signals but to a lesser extent than the US and China CDC primer combinations (see * in lines 1,3 and 5 in Figure 6b). However, primer dimer formation is seen in the gel image with the US CDC (line 1) and the Charite RdRp (line 5) primer pair (arrow), (see modified Figure 7).

Figure 7 taken from Jung et al. (Figure S5.):
(a) Melting curve analysis and (b) polyacrylamide gel image of PCR products with primer- probe sets that show positive signals in the NTC samples. M: DNA ladder; 1: NTC sample with 2019-nCoV_N1 (US CDC); 2: PCR product with 2019-nCoV_N1 (US CDC); 3: NTC sample with N (China CDC); 4: PCR product with N (China CDC); 5: NTC sample with RdRp_SARSr (Charité); 6: PCR product with RdRp_SARSr (Charité)



Conclusion:

The RdRp PCR from the Corman *et al.* publication produces less false positive amplification than the US and China CDC N1 and N PCR, however it still produces a very problematic amplification of “water only” which is a clear no-go for a PCR reaction intended for diagnostic use.

3. Etievant *et al.*

This citation also demonstrates poor results with the Charité E gene-assay and attributes this to primer contamination and primer dimers. Etievant *et al*. highlights the dimerization that can occur between E and RdRp gene-assays:

"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." [8]

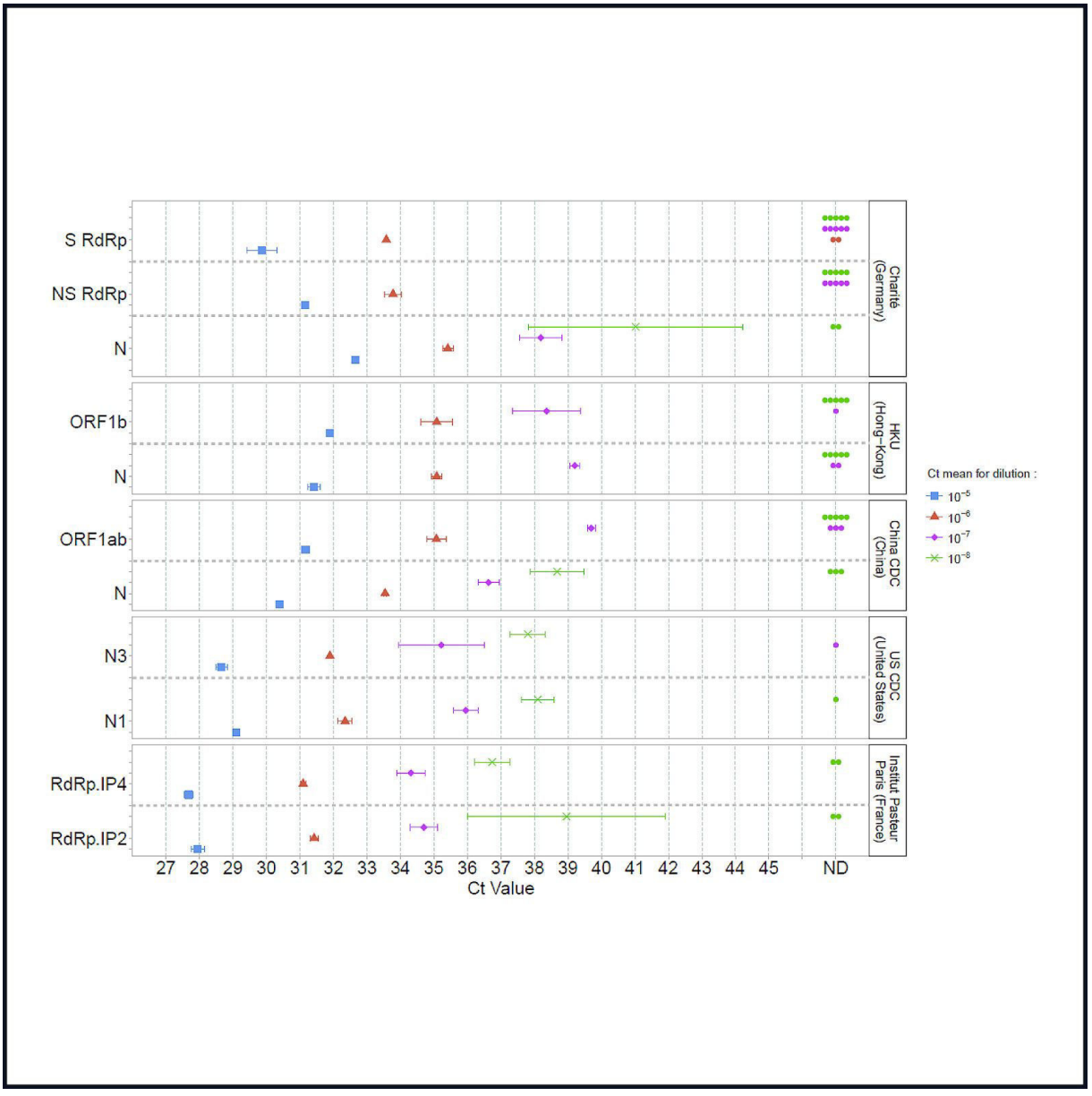
In theory, this should be a rare occurrence if labs are running singleplex assays without primer contamination, yet it is readily found in peer-reviewed literature with these exact assays and conditions by Etievant *et al*. Even with singleplex assays free of primer contamination, RdRp probe forms a hairpin and a self-dimer and this likely explains the reduced sensitivity of this assay (Figure 8).

The Etievant *et al.* study demonstrates that the CT values are in question as the Corman-Drosten paper did not disclose this important detail:

"*It is worth noting that the Charité assay was the first to be published at the early stage of the pandemic and has been widely used worldwide."*
[...]
"Of note, we did not apply the Ct cut-off values above, in which a sample would be considered negative, ***since such values were not provided*** *in the protocols made available by the referral laboratories."*
[...]
"As previously reported, we identified probable ***primer contamination*** *using N2 US CDC and E Charité, which prevented us from further evaluating their sensitivity and specificity." [8]*

These authors could not determine the sensitivity and specificity of these assays due to the flaws we explain in the retraction request. Known sensitivity and specificity are paramount to clinical diagnostics as described in Klement & Bandyopadhyay [9].

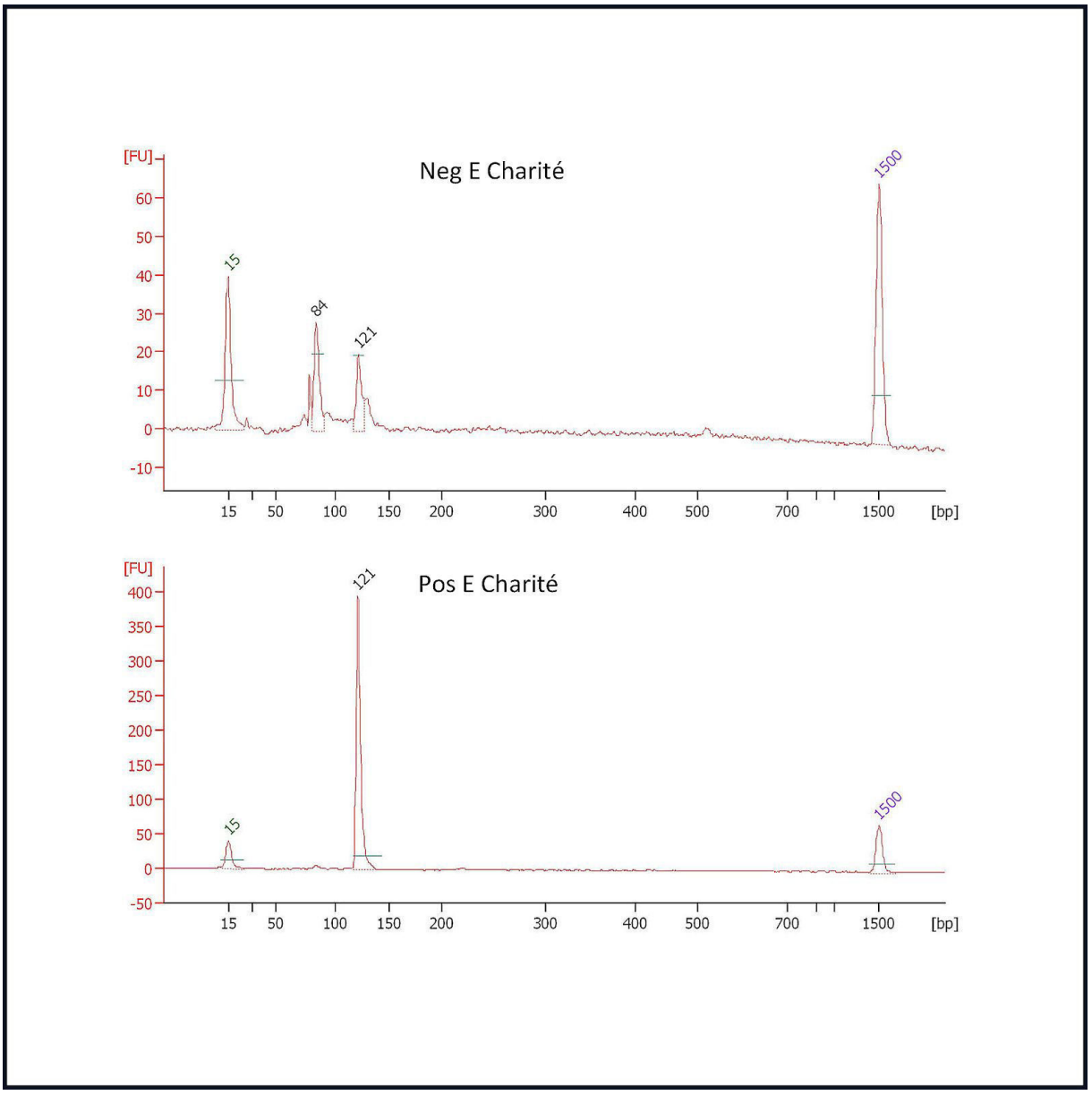
Figure 8 taken from Etievant *et al. (Figure 1)*:
Mean Ct values and standard deviations obtained using five PCR-based methods for SARSCoV-2 detection. Serial dilutions of SARS-CoV-2 cell culture supernatants were used and are represented by a single color (10−5 blue, 10−6 red, 10−7 pink, 10−8 green). A point in the ND (non-detected) column (Ct value axis) indicates a negative result for one replicate.



Upon exploration of the false positive signals obtained with the Corman *et al.* E-gene, the authors noted:

"For E Charité, negative samples showed two amplicons, one at 84 base pairs (bp) and one at 121 bp, whereas the positive sample only had one amplicon at 121 bp, which is close to the expected size of a specific amplification (Table 1). 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)." [8] (Figure 9)

Figure 9 taken from Etiviant et al. (Table 1): Charité assay targeting severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)



Conclusion:

The Corman *et al.* E-primer pair produces false negatives either due to contamination or to unspecific amplification.

4. Gand *et al.*

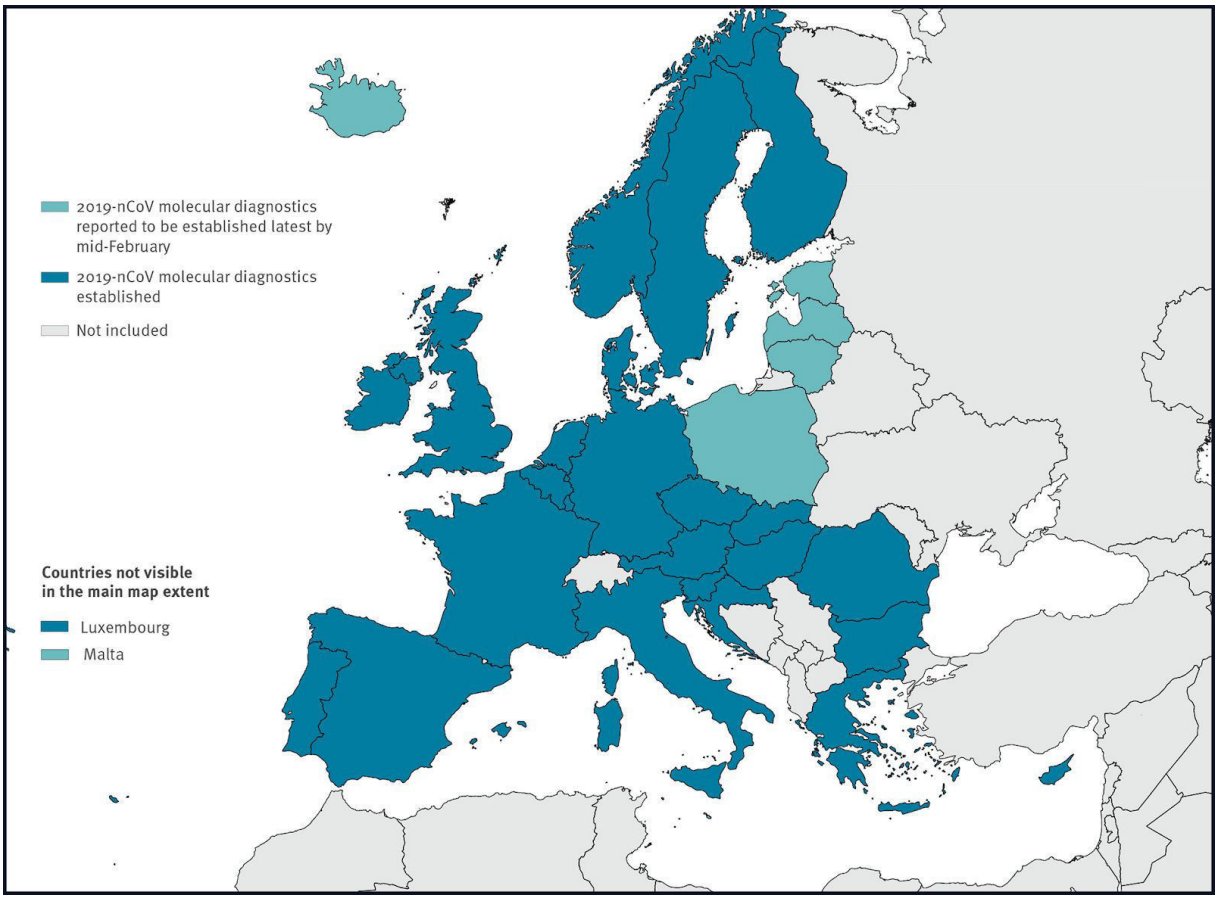
Gand *et al*. [10] notes that the Charité primers were the most widely used in Europe in the spring of 2020, referencing Reusken *et al*. published at the end of January 2020 at Eurosurveillance:

"*The RT-qPCR test developed by Corman and colleagues at Charité (Berlin) is the most widely used in Europe."*

A publication by Chantal Reusken and Marion Koopmans is referenced [52] (Figure 10). Both are co-authors of the Corman-Drosten-paper. Chantal Reusken is also on the editorial board of Eurosurveillance.

Further global use rates of the CD assays in the time frame January to December 2020 are not known and are difficult to deduce from the scientific literature. Since Charité Berlin did not claim any patent ownership for the invention, it is difficult to track usage with traditional royalty streams or estimates of revenues [11].

Figure 10 taken from Reusken *et al.* (Figure 2):
Status of availability of molecular diagnostics for novel coronavirus (2019-nCoV) in EU/EEA countries as at 29 January 2020 (n = 46 laboratories)[a]



As highlighted in our initial review, the authors (Gand *et al.*) mention that the false positives observed were predictable by in-silico analysis.

"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, and it was hypothesized that these SNPs present in almost all SARS-CoV-2 genomes could be the reason for this. As the utmost sensitivity is required for SARS-CoV-2 detection, especially when the viral load is low depending on the time and nature of the sampling, it might be proposed to correct such mismatches with the aim to potentially increase the sensitivity of Assay_2_RdRp-P1, Assay_2_RdRp-P2, Assay_8_RdRp, and Assay_10_E. The SNP present in the reverse primer of Assay_5_N was already corrected in a revised version of the protocol ***but has not yet been updated in the WHO*** *technical guidance."*

The authors point out that similar false positive results were predictable with their in-silico analysis and that the WHO has yet to address the errors in the Drosten primers.

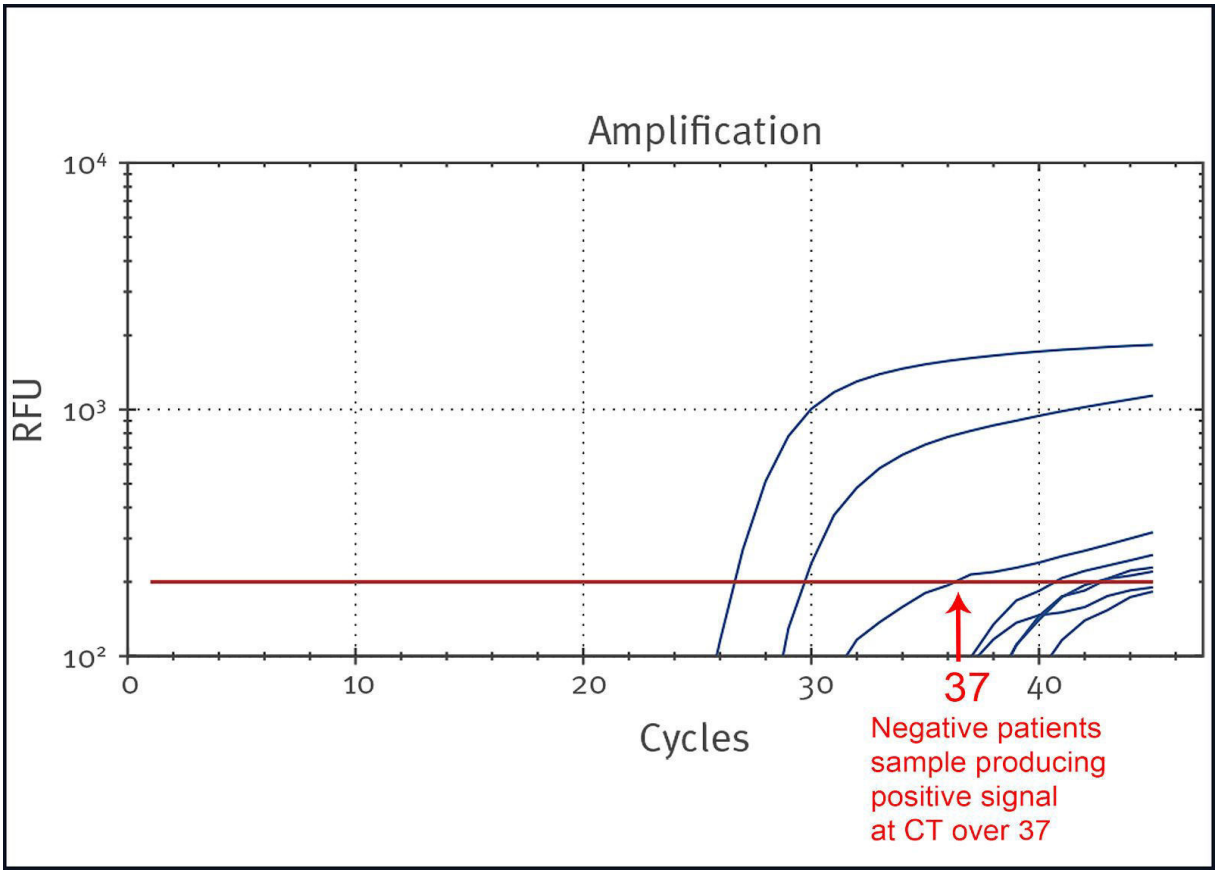
*"Interestingly, for Assay_2_RdRp-P2, similar false-positive results **as obtained in our in silico study** were obtained in the wet lab by Chan and colleagues, who detected SARS-CoV when using the probe P2 targeting the RdRp gene that is considered strictly specific to SARS-CoV-2. This indicates that our **in silico analysis** can be backed up by in vitro data."*

5. Konrad *et al.*

Konrad *et al.* report similar problems with false positive (FP) signals at high Ct. They report 61% FPs with their first test system. They improve upon this by changing their PCR master mix but still achieve a 5.1% FP rate with the improvement.

*"We found that the SARS-CoV E gene screening assay with the QuantiTect Virus +Rox Vial kit showed moderate to high amounts of unspecific signals in **late cycles in 61%** (451/743) of the tested patient samples and also of negative extraction and non-template controls (Table, Figure 2), which complicated the evaluation of the qPCR result. The RdRp assays were basically free from such unspecific signals in late cycles."* [12] (Figure 11, Figure 12)

Figure 11: taken from Konrad et al (Figure 2):
Example image of real-time RT-PCR curves of the gene assay with unspecific signals at late cycles, Bavaria, February 2020



RFU: relative fluorescence units.
Curves: 1: Wuhan coronavirus 2019 E gene positive control; 2: SARS-CoV Frankfurt 1 RNA positive control; 3,4,6,8: negative patient samples; 5: extraction negative control; 7: non-template negative control.

Signal is given in log scale with threshold = 200. PCR was performed with SuperScript III system and E gene primers and probe as published in [5]. Curves of positive controls (1 and 2) show expected sigmoid curves. Curves 3–6 show unspecific signals with increase above threshold. Curves below threshold were not considered as significant signals (7 and 8).

Figure 12 taken from Konrad et al.*(Table)*:
Comparison of two different one-step real-time RT-PCR systems with SARS-CoV-2 assays from Corman et al. [5] and a commercial test kit with kit-specific assays, Bavaria, February 2020

Real-time RT-PCR system	PCR efficiency (%)[a], linearity (R^2)	Limit of detection (copies/reaction)	Unspecific signals count in E gene assay in total[b]	Unspecific signals in E gene assay (%)[b]	Run time (hours)
QuantiTect Virus+Rox Vial kit (QIAGEN)	ND	ND	451/743 (75/126 NC, 376/617 patient samples)	60.7	1:50
SuperScript III One-step RT-PCR System with Platinum TaqDNA Polymerase (Invitrogen)	95 / 0,99[c]	50[c]	13/257 (2/38 NC, 11/219 patient samples)	5.1	1:28
RealStar SARS-CoV-2 RT-PCR kit 1.0 (Altona)	125 / 0,97[d]	10[d]	0/111 (0/38 NC, 0/73 patients samples)	0	2:15

NC: negative control samples; ND: not determined.

[a] $E = 10^{-1/slope} - 1$.

[b] Indicated counts and percentage values of unspecific background signals in the SARS-CoV E gene assay are based on the total number of tested patient samples as well as the negative extraction and non-template controls.

[c] Only for RdRp gene assays, tested with four replicates of SARS-CoV Frankfurt 1 RNA [6]; 10-fold serial dilutions were determined. For the E gene, the assay was not linear.

[d] Only for the E gene, tested with two replicates of synthetic Wuhan coronavirus 2019 E gene control and SARS-CoV Frankfurt 1 RNA each [6]; 10-fold serial dilutions were determined.

The authors conclude this is due to nonspecific signals from dimerisation of primers and probes as mentioned in our retraction request:

"Using commercial kits with optimised target regions and primer sequences (in the E gene and SARS-CoV-2-specific S gene) ruled out the unspecific signals completely. Hence, reasons for the observed ***unspecific signals may be dimerisation of primers*** *and probes and/or unspecific primer binding and polymerase activity in the targeted region of the E gene, probably also depending on thermal profile and cycler-specific differences, or most likely a combination of these factors."* [12]

6. Sethuraman *et al.*

Sethuraman *et al.* did not perform experiments themselves but instead refer to Nalla *et al.* in connection with the problematic Charité primers. They attribute this to the mismatch in the reverse primer:

"The sensitivities of the tests to individual genes are comparable according to comparison studies except the RdRp-SARSr (Charité) primer probe, which has a slightly lower sensitivity likely due to a mismatch in the reverse primer." [13]

7. Nalla *et al.*

Nalla *et al*. performed sensitivity tests with the three original Corman *et al.* PCR compared to the US CDC N genes and the RdRp of their own lab. Here, the E-gene test was very sensitive and the N-and RdRp gene PCRs showed reduced sensitivity compared to others.

"Assays using UW RdRp and Corman N-gene primer-probe sets have limits of detection (LODs) of about 790 viral genomic equivalents per reaction."
[...]
"*Assays using the Corman RdRp and E-gene sets were found to have LODs of about 316 viral genomic equivalents per reaction."*
[...]
"*Assays using the CDC N2 and Corman E-gene primer-probe sets were more sensitive than those using the CDC N1 and Corman RdRp sets and the BGI kit."* [24], (Figure 13)

Figure 13: Table reproduced from Nalla *et al.*

TABLE 2 Relative performance of SARS-CoV-2 detection assays using five different primer-probe sets[a]

Sample ID	CDC N1	CDC N2	CDC N3	Corman RdRp	Corman E-gene
SC5777	24.5	23.2	23.3	29.0	24.9
SC5778	30.2	30.6	30.1	34.8	31.9
SC5779	33.3	32.8	32.0	36.5	34.7
SC5780	14.6	13.7	13.9	19.2	15.1
SC5781	15.1	14.1	14.3	20.2	16.2
SC5782	21.8	20.9	21.0	26.9	22.6
SC5783	16.0	14.9	15.6	20.8	16.9
SC5784	36.0	35.6	Negative	Negative	35.4
SC5785	27.8	27.3	27.4	32.7	28.9
SC5786	23.9	24.0	24.3	29.4	25.6

[a]Cycle thresholds are displayed.

The Nalla *et al.* authors include a panel of other respiratory viruses in their PCR testing, however, results are mentioned for the CDC N1 and N2-primer probe sets only, not for the Corman *et al.* primer/probes combinations, despite a sentence in the discussion claiming:

"Of the seven different primer-probe sets and one testing kit that we evaluated, all were found to be highly specific with no false-positive results observed when assays were run on samples positive for a number of other respiratory viruses." [24]

8. Vogels *et al.*

Vogels *et al.* describe the errors in the RdRp-SARSr_R Charité primer with 99.8% mismatch frequency in SARs-CoV-2. This is due to the Corman-Drosten primer design being performed and verified on the basis of a non-relevant SARs-CoV-1 sample (Figure 14, Figure 15):

"Thus far, we detected 12 primer–probe nucleotide mismatches that had occurred in at least two of the 992 SARS-CoV-2 genomes. The most potentially problematic mismatch is in the RdRp-SARSr reverse primer, which probably explains the sensitivity issues with this set. Oddly, the mismatch is not derived from a new variant that has arisen, but rather that the primer contains a degenerate nucleotide (S, binds with G or C) at position 12, and 990 of the 992 SARS-CoV-2 genomes encode for a T at this genome position." [14]

Figure 14 taken from Vogels *et al*. (Table 2):
High-frequency primer and probe mismatches may result in decreased sensitivity for SARS-CoV-2 detectionTable

Table 2 | High-frequency primer and probe mismatches may result in decreased sensitivity for SARS-CoV-2 detection

Institute	Primer–probe	Primer–probe position 5′–3′	Genome position 5′–3′	Primer–probe nucleotide	Nucleotide in ref. genome[a] (RC)	Expected target nucleotide	Mismatch target in genomes[b] (frequency)
China CDC	CCDC-N-F	1	28,881	G	G (C)	C	T^{RC} (126/992; 12.7%)
	CCDC-N-F	2	28,882	G	G (C)	C	T^{RC} (126/992; 12.7%)
	CCDC-N-F	3	28,883	G	G (C)	C	G^{RC} (126/992; 12.7%)
	CCDC-ORF1-F	17	13,358	C	C (G)	G	A^{RC} (2/992; 0.2%)
	CCDC-ORF1-P	26	13,402	T	T (A)	A	C^{RC} (4/992; 0.4%)
Charité	E_Sarbeco_R	12	26,370	G	C (G)	C	T (4/992; 0.4%)
	RdRp-SARSr_R	12	15,519	S	T (A)	C or G	**T (990/992; 99.8%)**
HKU	HKU-N-F	4	29,148	T	T (A)	A	G^{RC} (5/992; 0.5%)
US CDC	2019-nCoV_N1-P	3	28,311	C	C (G)	G	A^{RC} (2/992; 0.2%)
	2019-nCoV_N1-R	15	28,344	G	C (G)	C	A (4/992; 0.4%)
	2019-nCoV_N3-F	8	28,688	T	T (A)	A	G^{RC} (39/992; 3.9%)
	2019-nCoV_N3-R	14	28,739	C	G (C)	G	T (4/992; 0.4%)

[a] Nucleotide (DNA form) found in the reference genome (NC_045512) and its reverse complement (RC). [b] Mismatch target is the disagreement between the expected target nucleotide and the nucleotide in the genome. Listed are mismatched nucleotides with primers and probes with frequency >0.1% in 992 genomes inspected in this analysis. The column at the far right highlights the various frequencies of mismatches, which would represent a mispairing following binding of the primers listed above. The high-frequency mismatch in the RdRp-SARSr reverse primer is highlighted in bold. A list of degenerate nucleotides incorporated into the primer and probe sequences can be found in Supplementary Table 4. Data used to make this table can be found in Source Data Fig. 4.

Vogels *et al.* further states:

"At 10^0 and 10^1 viral RNA copies µl–1, our results show that all primer–probe sets, except RdRp-SARSr and 2019-nCoV_N2, were able to partially detect (Ct < 40) SARS-CoV-2 from clinical sample." (Figure 15, Figure 16)

Figure 15 taken from Vogels *et al*. (Fig.1):

Analytical efficiency and sensitivity of the nine primer–probe sets used in SARS-CoV-2 RT–qPCR assays. *a,b, Mean Ct values for nine primer–probe sets and a human control primer–probe set targeting the human RNase P gene tested for two technical replicates with tenfold dilutions of full-length SARS-CoV-2 RNA (a) and pre-COVID-19 nasopharyngeal swabs spiked with known concentrations of SARS-CoV-2 RNA (SARS-CoV-2 RNA-spiked mocks (b)). The CDC human RNase P (RP) assay was included as an extraction control. c,d, From the dilution curves in a,b, PCR efficiency (c) and y-intercept Ct values (measured analytical sensitivity) (d) were calculated for each of nine primer–probe sets. Symbols depict sample type: squares represent tests with SARS-CoV-2 RNA and diamonds represent SARS-CoV-2 RNA-spiked mock samples. Colours denote the nine tested primer–probe sets. Dashed lines indicate 90% PCR efficiency (c) and the detection limit (d).*

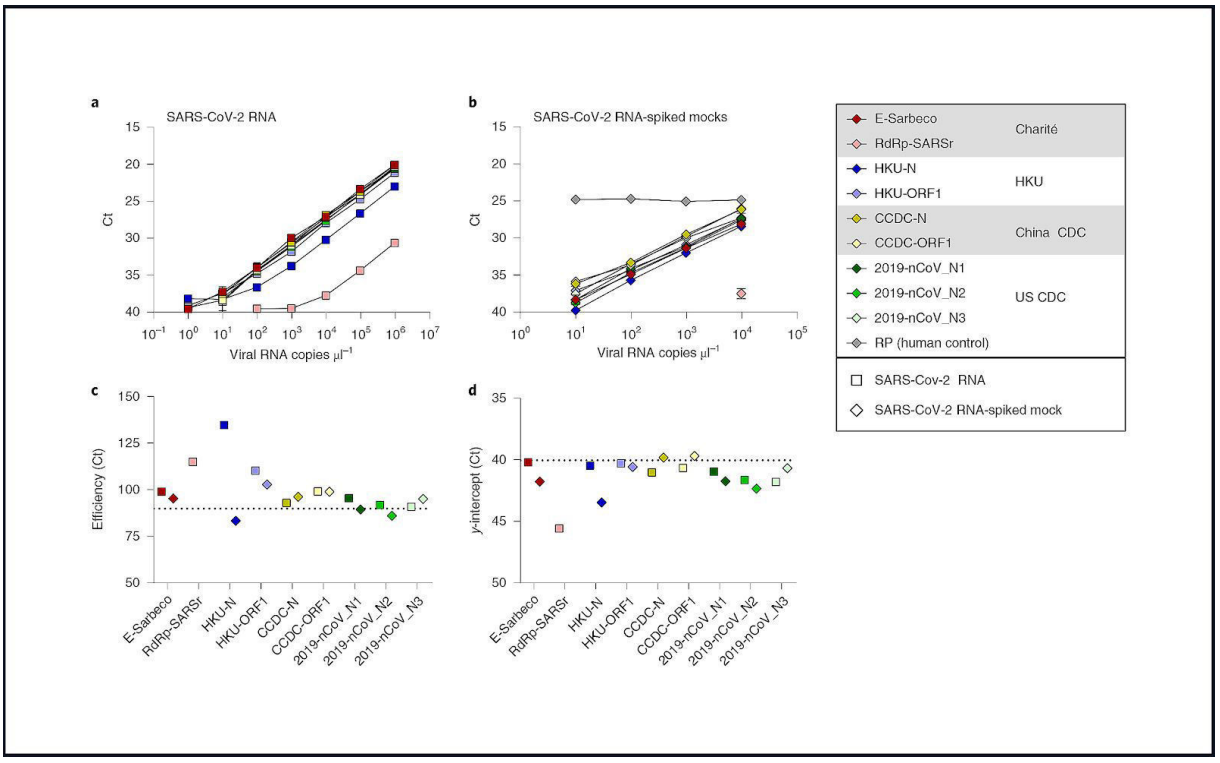
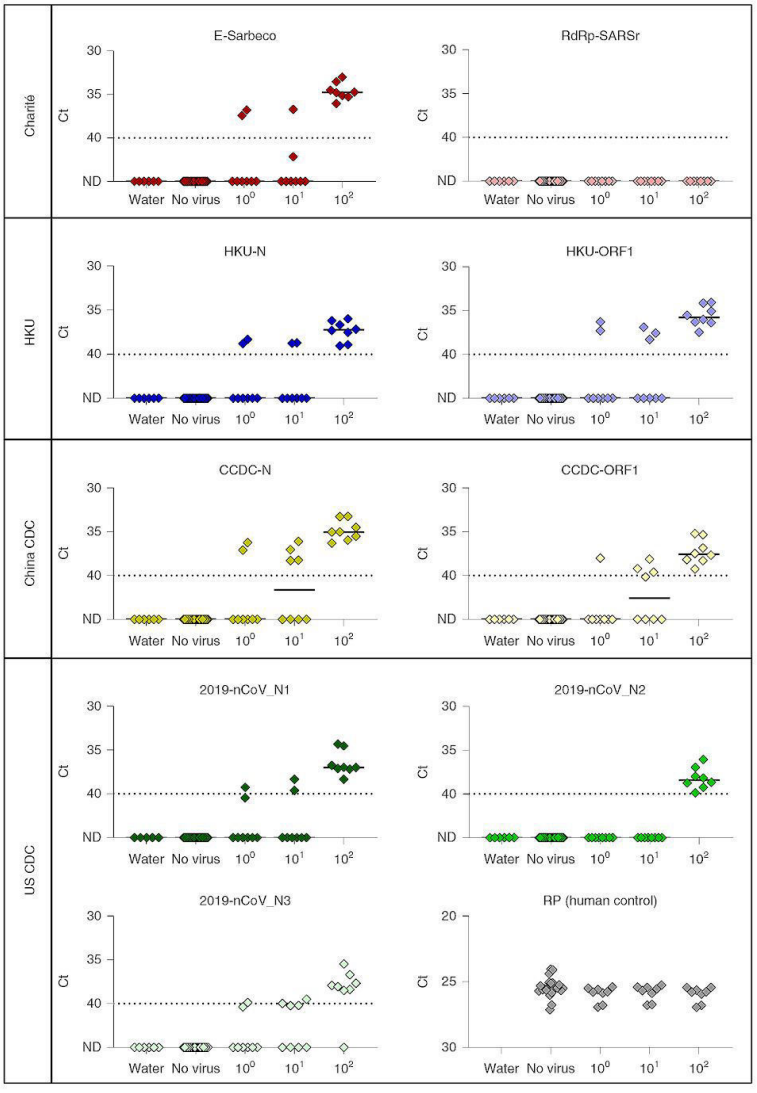


Figure 16 taken from Vogels *et al*. (Fig.2):

Comparison of analytical sensitivity of SARS-CoV-2 primer–probe sets using pre-COVID-19 nasopharyngeal swabs.

The lower detection limit of nine primer–probe sets, as well as the human RNase P control from RNA extracted from nasopharyngeal swabs collected in 2017 spiked with known concentrations of SARS-CoV-2 RNA. Each primer–probe set was performed using 24 technical replicates of pooled-swab RNA without spiking SARS-CoV-2 RNA ('No virus'; six replicates with four independent pools each of four swabs) and eight replicates (two replicates with four independent pools each of four swabs) spiked with 100–102 viral RNA copies µl–1 of SARS-CoV-2 RNA. ND, not detected. Solid lines indicate the median and dashed lines indicate the detection limit.



9. Kuchinski *et al.*

Kuchinski *et al.* [15] also demonstrate the errors in the RdRp assay, with 99.6% samples having a mismatch sequence as described in Vogels *et al*. [14], (Figure 17). This was also raised by Pillonel *et al*. [16] and this particular correspondence letter can be found now attached to the Corman-Drosten manuscript as an erratum at Eurosurveillance.

Figure 17 reproduced from Kuchinski *et al*. (Table 2):
Frequency of mismatches between 15,001 SARS-CoV-2 genome sequences and 15 sets of oligonucleotides from early lab developed tests. The Charite group - RdRP is shown here only.

Assay	0 mismatches	1 mismatches	2 mismatches	3+ mismatches
Charité group - N	98,9%	0.9%	0.0%	0.1%
Charité group - RdRP	0.0%	99.6%	0.3%	0.0%
Charité group - E	99,6%	0,2%	0,1%	0,0%

Under section 3.3 it is stated:

*"**Pervasive single nucleotide mismatches in assays from Charité Group and Japan NIID:** Two sets of oligonucleotides had mismatches against all 15,001 SARS-CoV-2 reference genomes in our dataset: the Charité group's RdRP gene assay and the Japan NIID's N gene assay."*

10. Ratcliff *et al.* PrePrint

Ratcliff *et al*. is still in PrePrint form but also explains the underperforming primer sequences circulated by the WHO and recommended by the Corman Drosten protocol.

*"Unexpectedly, the performances varied substantially depending on the detection method and target assayed, underpinning the need for in-house validation and optimization. The result also **challenges the notion that Ct values presented without context could be an informative metric for the progression of disease** and can be compared across different*

amplification techniques and laboratories."
[...]
"The Charité RT-PCR was based upon previously described primer/probes for the RdRP gene but with modifications to the antisense primer to ensure complete sequence complementarity with SARS-CoV-2 sequences."
[...]
"All primers and probes for the Charité and CDC N1 PCRs were obtained from ATDBio. All primer sequences and working concentrations are available in Table 1." [17] (Figure 18)

Figure 18 taken from Ratcliff *et al.* (Table 1):
Primer and Probe Sequences for Nested PCR and RT-qPCR

PCR Assay	Primer Name	Sequence	Reaction concentration
Nested PCR	nF1	AYTCAATGAGTTATGAGGAYCAAGATGC	400 nM
	nR1	GACATCAGCATACTCCTGATTWGGATG	400 nM
	nF2	TAGTACTATGACMAATAGACAGTTYCATC	500 nM
	nR2	CCTTTAGTAAGGTCAGTCTCAGTCC	500 nM
Charité RdRP	RdRp_SARSr F	GTGARATGGTCATGTGTGGCGG	600 nM
	RdRp_SARSr P2	FAM CAGGTGGAACCTCATCAGGAGATGC BHQ	100 nM
	RdRp_SARSr R	CAAATGTTAAARACACTATTAGCATA	800 nM
CDC N1	2019-nCoV_N1-F	GACCCCAAAATCAGCGAAAT	500 nM
	2019-nCoV_N1-P	FAM-ACCCCGCATTACGTTTGGTGGACC BHQ	125 nM
	2019-nCoV_N1-R	TCTGGTTACTGCCAGTTGAATCTG	500 nM

11. Jaeger *et al.*

Jaeger *et al.* characterize the primer dimers observed in these protocols and how these can create signals even with Taqman or probe hydrolysis based methods. This is a common complaint about our initial retraction letter. While we pointed out the primer dimer potential, most colleagues falsely assumed this was only a problem with SYBR green based qPCR. They are correct to point this out as SYBR green is much more prone to Primer-Dimer signals since its signal is derived from sequence-independent intercalating dyes. This non-specific amplicon labeling method usually requires a High Resolution Melt (HRM) analysis to confirm the target amplicon size. SYBR green based methods require this HRM step to confirm the specificity of the intercalating dye signal. Taqman or Hydrolysis probe based methods achieve this specificity by labelling a sequence-specific probe that is independent of the PCR primers. Jaeger *et al*. demonstrate probe hydrolysis can also occur as a result of primer dimers or primer-probe-background interactions in Taqman-based assays. Jaeger *et al.* even run gel electrophoresis on the samples with spurious qPCR signals and find primer dimers or other nonspecific signals. They cite Konrad and Pillonel as support for this.

"The apparent occurrence of dimerization does not appear to be exclusive to nucleocapsid targets. ***Unspecific signals in the late cycles of the envelope protein gene (E target) assay using the Charité protocol*** *(Konrad et al., 2020) and a mismatch of primer sequences (Pillonel et al., 2020) have been reported recently. The scientific community is discussing the technical limitations of the current SARS-CoV-2 RT-qPCR protocols (Marx, 2020) and their optimization is still underway."* [18] (Figure 19)

"*However,* ***fluorogenic probe-based reactions are not supposed*** *to be influenced by dimerization in the N2 primers–probe and/or primer–primer from the CDC RT-qPCR recommended protocol used for SARS-CoV-2 diagnosis. Won et al. (2020) found unspecific amplifications when using the N2 and N3 primers–probe sets and then proposed an alternative primers–probe panel for the nucleocapsid target*." [18] (Figure 19)

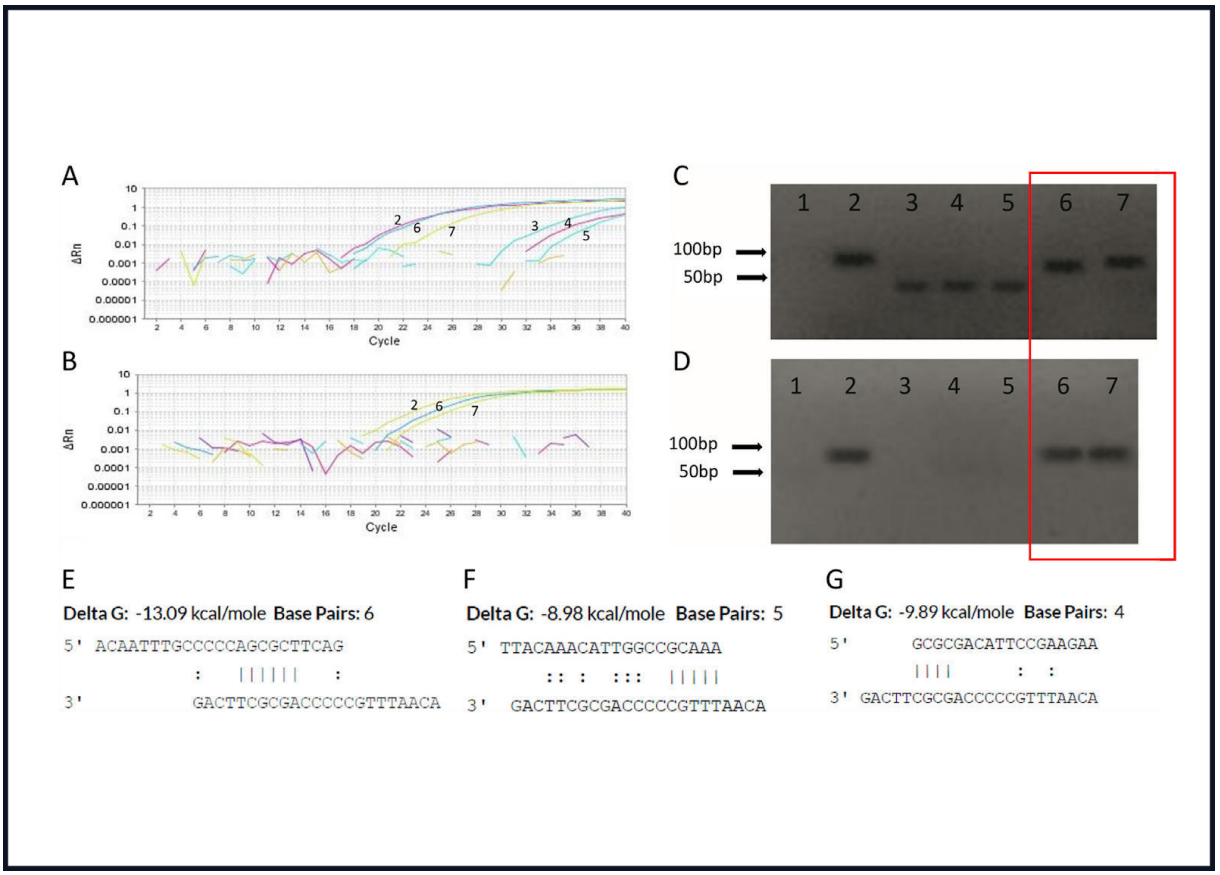
Note their specific comment that speaks of fluorogenic probe-based assays typically not generating signals but with these promiscuous primers they generate false positive signals**.**

Jaeger *et al.* also concludes:

"Finally, we recommend that RT-qPCR users adjust primers–probe and magnesium concentrations, the duration of the reverse transcriptase step, and the thermal cycle

temperature, independent of the master mix kit used, to minimize dimer formation and to avoid extensive test repetition and the waste of resources."

Figure 19 taken from Jaeger *et al.* (Figure 1):
Dimerization during RT-qPCR with the CDC N2 primers–probe set. Amplification plots of initial (A) and optimized (B) RT-qPCR conditions. Dimer formation can be visualized by the late signal produced in 'not detected' samples (curves 3, 4, and 5). Gel electrophoresis of initial (C) and optimized (D) RT-qPCR conditions. Dimers appear as diffuse bands (lanes 3, 4, 5) at the bottom of the gel (PCR products <50 bp). Partial sequence homologies between probe–probe (E), primer F–probe (F), and primer R–probe (G) estimated by OligoAnalyzer v.3.1. Key: 1 = no-template control (NTC); 2 = 2019-nCoV_N Positive Control (IDT); 3, 4, 5 = 'not detected' samples, 6, 7 = positive samples.



12. Khan *et al.*

Khan *et al.* even discuss the propagation of an erroneous protocol having been circulated by the WHO and articulate the need to re-assess the suggested primers for SARS-CoV-2 RT-qPCR detection:

*"Despite the ability of single mismatches to be tolerated, it is important to consider that **mismatches need to be corrected** if found in most of the viral sequences available. For example, the reverse primer of Charité-ORF1b shows a mismatch with all the viral sequences (a total of 17 002). This mismatch has also been observed in 990 viral sequences along with the lower sensitivity of this assay in a recent preprint."*
[...]
"*However, some of the assays have not been reassessed in the light of the risk of mutations during viral evolution. Based on the analysis of 17 027 viral sequences, this study demonstrates the presence of mutations/mismatches in the primer/probe binding regions of some published assays (table 3). Sequences adjustments to these primers/probes need to be assessed experimentally using viral strains or nucleic acid coupled with subsequent experimental performance using clinical samples."* [19]

13. Opota *et al.*

Opota *et al*. [20] also abandon Charité's RdRp assays claiming:

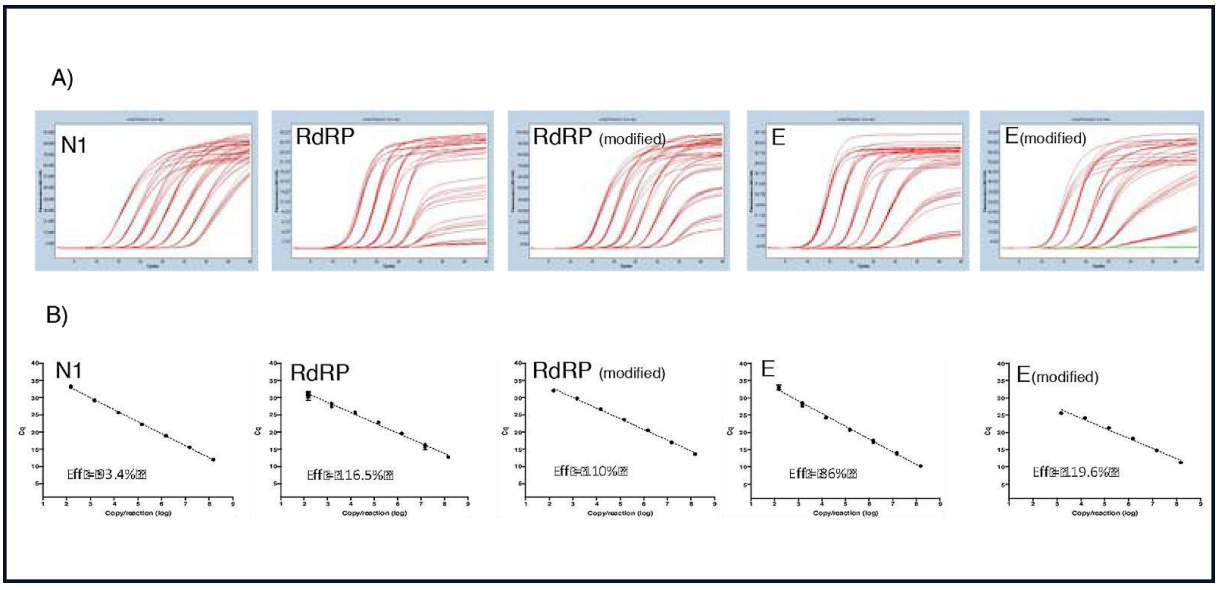
"*Future studies should also include the comparison of in-house RdRP RT-PCR with commercial RT-PCR. Indeed, this comparison was not achieved as the RdRP RT-PCR needed further optimization based on recent publication that elucidated the reason of the limited sensitivity as the difference in the melting temperature of the forward and reverse primers of the initial PCR of Corman and colleagues (Corman and Drosten 2020; Muenchhoff et al. 2020; Pillonel et al.2020)."*
[...]
"*The RT-PCR targeting the RdRP gene and the N-gene were also introduced according to Corman and colleagues but showed a significantly reduced sensitivity **requiring further optimization and was not used for this comparison** (Pillonel et al. 2020)."*

14. Barra *et al*. (Preprint)

Barra *et al.* also make note of the reduced sensitivity of the RdRp assay. They test this against a modified RdRp assay and are careful to point out that the original Corman-Drosten primer set was never tested on real SARs-CoV-2 but on in-vitro transcribed SARs-CoV-2 RNA (IVT). In-vitro transcribed RNA does not contain the subgenomic RNA and therefore represents an ideal circumstance that isn't reflective of real world samples.

"The sensitivities observed in this study were slightly different than the described for RdRP (3.6 copies per reaction) and E (3.9 copies per reaction) original description, where the authors used the in vitro transcribed SARS-CoV-2 RNA directly in the reaction." [21], (Figure 20);

Figure 20 taken from Barra *et al.* (Preprint, Figure 2):
Assays limit of detection determination. N1 and RdRP (modified) showed better LOD. A) Raw data and B) Probit regression analysis (inserted unit values are copies/reaction).



15. Santos *et al.*

Santos *et al.* [22] aligned different primer / probe pairs against a broad collection of SARS CoV-2 gene sequences derived from Brazil. Here, they also report mismatches in the Charité's E primer sets:

"The French nCoV_IP4 and Chinese CN-CDC-E assays demonstrated total identity to their motives. The other assays, nCoV_IP2, CN-CDC-ORF1ab, Charité-E, and E_Sarbeco showed low frequency of errors, such as 1 to 2 bp mismatches."

"The assays 2019-nCoV (N1, N2, and N3), NIH-TH_N, nCoV_IP2, CN-CDC-ORF1ab, Charité-E, and E_Sarbeco, presented mismatches located in the 5' or central portion of their primers when aligned with the Brazilian viral genomes." [22] (Figure 21, Figure 22)

Figure 21 taken out of Santos *et al*. (Table 1):
List of analyzed assays by targets, frequency and location of mismatches. Each assay below includes three components, 2 primers and 1 probe. Both can be susceptible to matching errors.

Assays/Origen	Target	Total frequency of mismatches	Mismatches at 3' or 5' portion
US-CDC-N1/US-CDC	N	3/177	5' and 3'
US-CDC-N2/US-CDC	N	2/177	5'
US-CDC-N3/US-CDC	N	3/177	5' and 3'
NIID_2019-nCOV_N/Japan	N	0/177	-
N_Sarbeco/Japan	N	1/177	3'
CN-CDC-N/China	N	151/177	5'
HKU-N/Hong Kong	N	103/177	5' and 3'
NIH-TH_N/Thailand	N	2/177	5'
Corman-N/Germany	N	1/177	3'
nCoV_IP2/France	ORF1ab	1/177	5'
nCoV_IP4/France	ORF1ab	0/177	-
CN-CDC-ORF1ab/China	ORF1ab	2/177	5'
Charité-E/Germany	E	2/177	5'
CN-CDC-E/Germany	E	0/177	-
E_Sarbeco/France	E	2/177	5'

Note that the Chinese and Hong Kong assays for the N gene have many mismatches compared to the others. ORF1ab and E targets are less frequent in 3 'mismatches.

16. Anantharajah *et al.*

Anantharajah *et al.* described the evaluation of the primer/probe sets designed by the US CDC and Charité/Berlin (which is Corman et al) to detect clinical cases which were defined as "COVID-19 cases by chest CT". In this work (Figure 22), the RdRp assay is once again the worst performing assay (lowest rate of positive detection, highest Ct value) amongst all tested, which was discussed to be based on the:

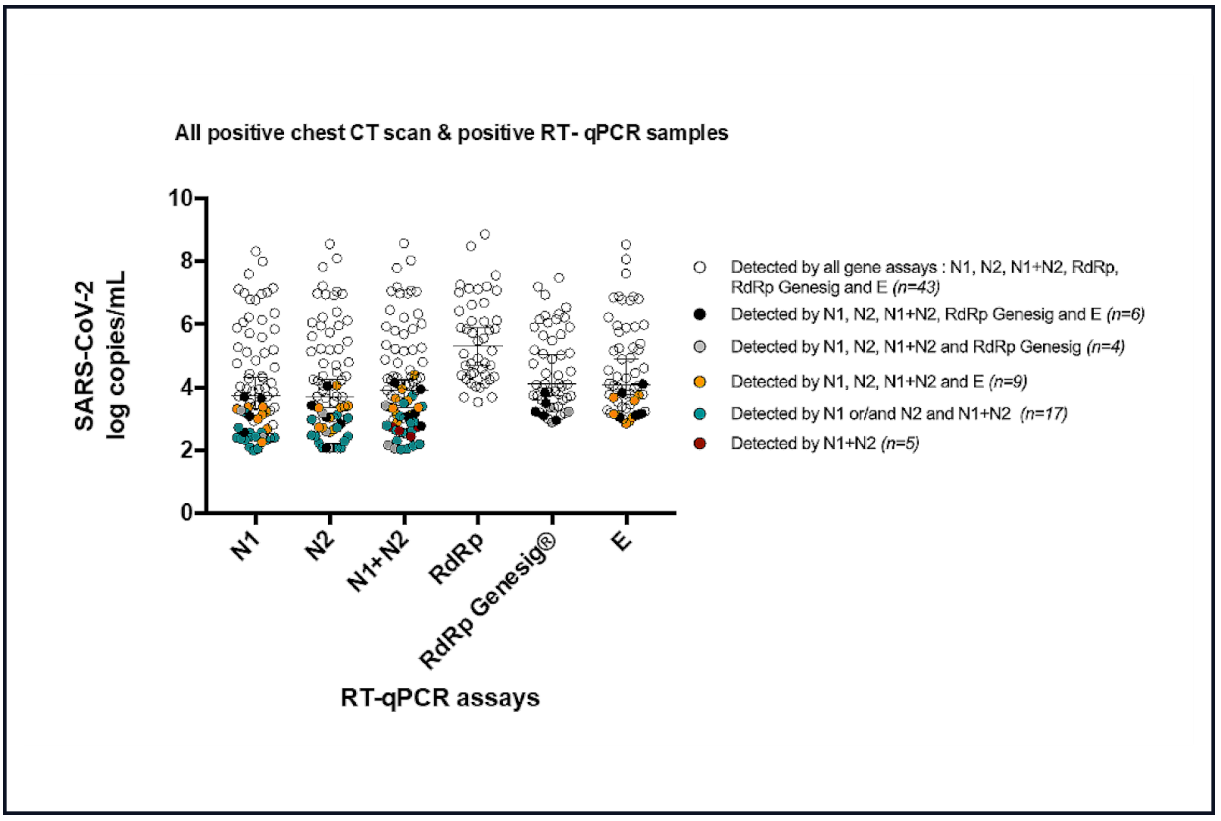
"Incorrect degenerate base S at position 12 that binds with G or C while all SARS-CoV-2 analyzed sequences encoded for a T at this position [...]. This mismatch would not be derived from a new variant but rather due to the initial oligonucleotide design allowing to amplify SARS-CoV, bat-SARS-related CoV and SARS-CoV-2-genomes." [23]

"Among them, the United States Center for Disease Control (US CDC) recommended two nucleocapsid gene targets (N1 and N2) [3] while the German Consiliary Laboratory for Coronaviruses hosted at the Charité in Berlin (Charité/Berlin) recommended first line screening with the envelope (E) gene assay followed by a confirmatory assay using the RNA-dependent RNA polymerase (RdRp) gene, even before the first COVID–19 cases appeared in Europe. At the time of data submission 295 molecular assays are commercially available or in development for the diagnosis of COVID–19 and most of them use these recommended gene targets alone or in combination."
[...]
"*We observed notable mismatches in regions targeted by the primers/probe sets which might affect RT-qPCR assays performance depending on their location and the nature of the substitution."* [23]

The authors further discuss:

"The findings highlight substantial differences in sensitivity for the primer/probe sets when comparing under the same conditions. Indeed, N1 and N2 assays stand out in comparison with the E and RdRp assays for the detection of low-level viral loads. Furthermore, positive E and negative RdRp results were obtained in 15 cases. We may therefore question the need of confirmatory testing following an initial positive test according to the Charité/Berlin protocol, resulting in turnaround time delay and increased workload." [23]

Figure 22 taken from Anantharajah et al. (Figure 2):
Comparison of the viral load detected by the six RT-qPCR assays among the positive nasopharyngeal swabs (n = 84). The viral load is expressed in log copies/mL and each clinical sample is represented by a circle. The white circles represent clinical samples detected by all RT-qPCR assays while colored circles represent samples not detected by the six assays. Bars represent the median and 95% Condence Interval



17. Dahdouh *et al*

In a letter to the Editor of J. Infect., Dahdouh *et al.* highlight the Ct variance seen in the internal controls that target human DNA concurrent with SARS-CoV-2 detection (Figure 21).

As a conclusion, they point out:

"A full characterization of the linear ranges and a calibration using standards should be done for every different target and primer/probe design." [25]

The calibration and internal controls are missing completely in the Corman *et al.* PCR design.

Given the numerous examples presented of FP and FN generation with the quickly designed Corman-Drosten primers, there is a final intellectual challenge which this assay presents. Unlike most other SARs-CoV-2 qPCR assays, the Corman-Drosten assay lacks any internal control. The lack of such controls makes any measurement with the assay exposed to a significant source (4 logs) of variability as there is no reference to interpret the viral loads, which cannot be determined from Ct values without such reference to an internal control. Dahdouh *et al.* highlight the Ct variance seen in the Internal Controls that target human DNA concurrent with SARS-CoV-2 detection (Figure 23).

Figure in Dahdouh *et al.* demonstrates the Ct variance of Internal Control (IC Ct) on the Y axis compared to SARS-CoV-2 N gene Ct variance. Samples with high IC Ct represent poor patient sampling as too little human DNA is present to enable effective sample collection. The relative viral load can possibly only be estimated with reference to sampling efficiency, e.g. the IC signal.

Analysis of the SARS-CoV-2 Ct values obtained using a commercial RT-qPCR assay (Vircell) in a set of clinical samples. A) Cts of the Internal Control RNA plotted against the SARS-CoV-2 N gene Cts (r2 = 0.004).

Direct Link to Figure:
https://els-jbs-prod-cdn.jbs.elsevierhealth.com/cms/attachment/92b776fc-71d1-450e-9ede-1e08c9768393/gr1.jpg

This demonstrates that the patient sampling and DNA/RNA purification steps can alter the RNA/DNA yield 1,000-10,000 fold (10-13 Ct's). This is an important variance as the world debates 33 vs 37 Ct for calling patients infectious. If one can not measure sampling variance and normalize for this, one can't offer a rational Ct threshold upon which to classify a patient as infectious.

"The plot shows an inverse linear correlation, which is expected because Ct values reflect, indeed, viral loads, but the dispersion of the data may reach up to four log units (ten thousand-fold) for any given Ct (black arrow)." [25]

Normalizing for this 13 Ct variance cannot be done with the Corman-Drosten primer set as it does not contain a human genome target amplicon (RNaseP Internal Control). So not only does the protocol lack a description of which Viral Ct to call a positive, it doesn't have a human internal control to normalize for the 10,000 fold variance in nucleic acid sampling. This is very much frowned upon in clinical diagnostics. Incorporating human ICs requires

benchmarking to viral standards that are identical to the target virus (not distant relatives from bats or SARS). (Figure 24)

Figure 24: CDC guidelines for use of Internal controls from the RNase P gene. CDC: 2019-Novell Coronavirus (2019-nCoV) Real-Time RT-PCR Diagnostic Panel. CDC-006-00019, Revision 05. 13.07.2020

2019 nCoV_N1	2019 nCoV_N2	RP	Result Interpretation[a]	Report	Actions
+	+	±	2019-nCoV detected	Positive 2019-nCoV	Report results to CDC and sender.
If only one of the two targets is positive		±	Inconclusive Result	Inconclusive	Repeat testing of nucleic acid and/or re-extract and repeat rRT-PCR. If the repeated result remains inconclusive, contact your State Public Health Laboratory or CDC for instructions for transfer of the specimen or further guidance.
-	-	+	2019-nCoV not detected	Not Detected	Report results to sender. Consider testing for other respiratory viruses.[b]
-	-	-	Invalid Result	Invalid	Repeat extraction and rRT-PCR. If the repeated result remains invalid, consider collecting a new specimen from the patient.

[a]Laboratories should report their diagnostic result as appropriate and in compliance with their specific reporting system.
[b]Optimum specimen types and timing for peak viral levels during infections caused by 2019-nCoV have not been determined. Collection of multiple specimens from the same patient may be necessary to detect the virus. The possibility of a false negative result should especially be considered if the patient's recent exposures or clinical presentation suggest that 2019-nCoV infection is possible, and diagnostic tests for other causes of illness (e.g., other respiratory illness) are negative. If 2019-nCoV infection is still suspected, re-testing should be considered in consultation with public health authorities.

RNase P (Extraction Control)

- All clinical samples should exhibit fluorescence growth curves in the RNase P reaction that cross the threshold line within 40.00 cycles (< 40.00 Ct), thus indicating the presence of the human RNase P gene. Failure to detect RNase P in any clinical specimens may indicate:
  - Improper extraction of nucleic acid from clinical materials resulting in loss of RNA and/or RNA degradation.
  - Absence of sufficient human cellular material due to poor collection or loss of specimen integrity.
  - Improper assay set up and execution.
  - Reagent or equipment malfunction.
- If the RP assay does not produce a positive result for human clinical specimens, interpret as follows:
  - If the 2019-nCoV N1 and N2are positive even in the absence of a positive RP, the result should be considered valid. It is possible, that some samples may fail to exhibit RNase P growth curves due to low cell numbers in the original clinical sample. A negative RP signal does not preclude the presence of 2019-nCoV virus RNA in a clinical specimen.
  - If all 2019-nCoV markers AND RNase P are negative for the specimen, the result should be considered invalid for the specimen. If residual specimen is available, repeat the extraction procedure and repeat the test. If all markers remain negative after re-test, report the results as invalid and a new specimen should be collected if possible.

18. Poljak *et al.*

The RdRp (RNA-dependent RNA polymerase) gene is a synonymous nomenclature. This enzyme is encoded by the nsp12 gene which is part of ORF1. RdRp is the cleavage product of the polyproteins 1a and 1ab from ORF1a and ORF1ab [43,53]. There is a high degree of conservation among RNA-dependent RNA polymerases of different RNA viruses which explains its lack of specificity to SARS-CoV-2.

Roche replaces the RdRp Corman primer with a more specific primer pair for SARS-CoV-2 called ORF1, also includes an Internal Control to monitor the sample preparation variance and also implements an enzymatic decontamination process (UDG) to reduce false positives. Four false positives are evident in the original Corman paper. The authors justify these false positives as 'user error' but since they lack the correct controls, this cannot be discerned from the information published and is a false conclusion derived from the data provided.

"The test utilizes RNA internal control for sample preparation and PCR 167 amplification process control. Uracil-N-glycosylase is included in the PCR mix to destroy 168 potential contaminating amplicons from previous PCR runs." [40]

The last paragraph of the results section states:

"*After extensive evaluation, our laboratory implemented LightMix-based SARS-CoV-2 testing on 17 January 2020."* [40]

This manuscript also sheds light on the timelines of disclosure for this test. Slovenia already had the TIB Molbiol LightMix earlier than January 17 2020, a period when no case of the "new virus" was even documented in Europe. Further, we can also conclude that TIB Molbiol (Olfert Landt) distributed those PCR kits with the Corman Drosten primers and probes at least one week before they submitted the original manuscript describing the protocol-design to Eurosurveillance, and presumably in parallel they were also sending out the protocol to the WHO.

Summary of the Poljak Methods:

- A) LightMix Modular SARS and Wuhan CoV E-gene kit and RdRp gene kit were used, the protocol followed the CormanDrsoten protocol, Ct values above 37 were considered negative.
- B) Cobas 6800 SARS-CoV-2 testing for the ORF1 gene and the Sarbeco E gene

Results:
First test (in-house panel):
2 of 217 samples were excluded from analysis due to invalid cobas results;
3 of 63 samples which were positive with the LightMix were negative by cobas;
1 of 152 samples which were negative by LightMix were found positive by cobas;

211/215 results were identical;

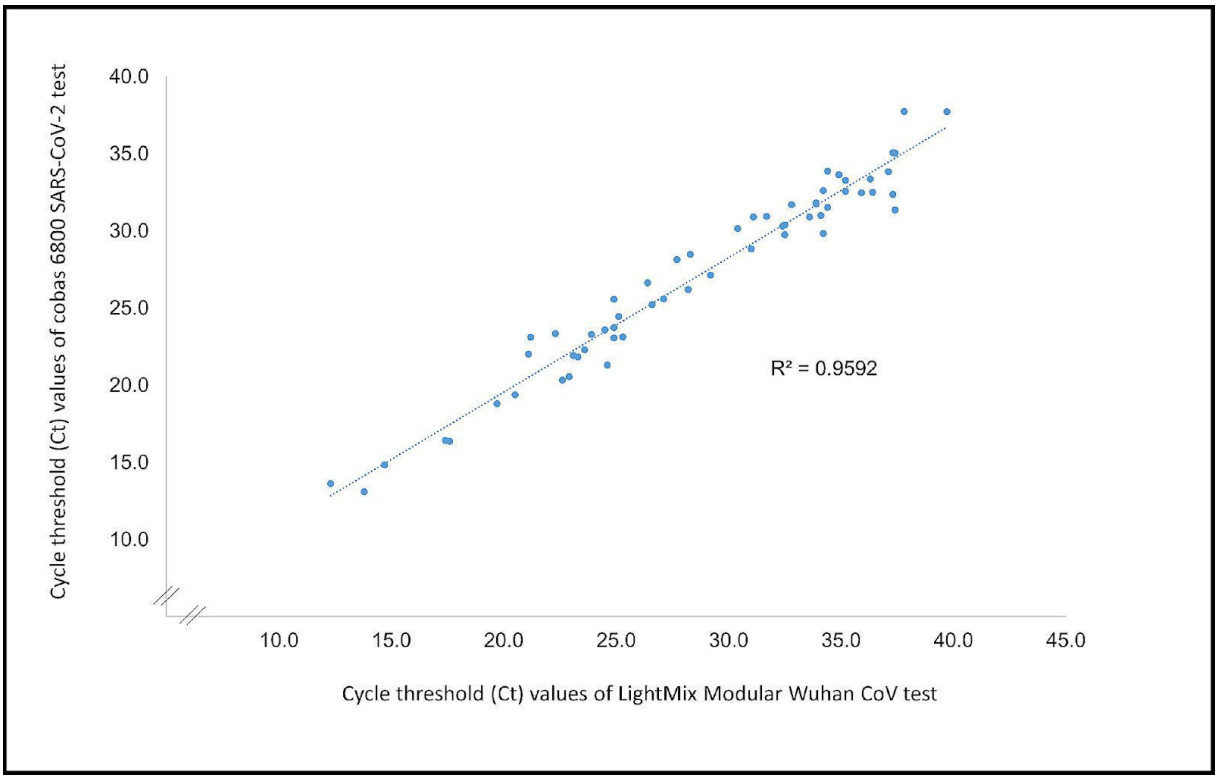
Second test (prospective comparison).
1 of 502 samples was excluded from analysis due to invalid cobas results
2 of 438 samples which were negative by LightMix were found positive by cobas

A correlation is shown in Fig 1 (Figure 25) for the positive samples in relation to the CT values of the RdRp gene and the ORF-1 gene.

Of note: Fig 1 in Poljak *et al.* does not show the correlation for the E-Gene, neither is this crucial data shown in the results or in the discussion section: the relevant data here is simply missing. About 28 samples had a Ct higher than 35 for the ORF-1/RdRp gene

Figure 25 taken from Poljak et al. (FIG 1):

FIG 1: Correlation between cycle threshold (Ct) values obtained by LightMix Modular Wuhan 406 CoV (RdRP gene – SARS-CoV-2 specific target) and cobas 6800 SARS-CoV-2 (target 1 – ORF1 – 407 SARS-CoV-2 specific target) in the prospective head-to-head evaluation performed on 502 408 samples. Ct values for the LightMix assay were always set to 0.1 normalized reporter dye 409 intensity (delta Rn). Linear regression of the Ct values was performed using samples positive for 410 SARS-CoV-2 by both diagnostic approaches (n = 63). The r² correlation value is indicated.



19. Boutin *et al.*

The authors compared an in-house test following the E-gene primers and probes of the Corman Drosten-protocol according to their publication with the Roché Cobas SARS-CoV-2 test, spanning the targets ORF1 and PAN-Sarbeco E gene [41].

Methods:

Additionally the Abbot real time SARS-CoV-2 test was used to clear discrepant results. Detection limit was quoted as 180 viral RNA copies per ml with the in-house test and 23 viral RNA copies per ml the Roché Cobas test.

Samples: 377 routinely collected nasopharyngeal/oropharyngeal swabs.

121 of those: no symptoms
132 symptomatic (no further definition is given on what type of symptoms)
124 without information

Results:

In-house E-gene: 281 of 377 samples were found positive ("detected") and 96 negative ("non detected"). This means a rate of positive samples of 74% .

Cobas: 301 samples were found positive and 76 negative (rate of positive samples: 80%);

Note: since at least 124 of the 377 samples were from asymptomatic patients, the rate of positivity is remarkably high.

Even if all individuals for whom no information is available were symptomatic, in total 256 symptomatic persons (68% of all) were tested, which means that from the defined asymptomatic persons, 25 (equals 21% with the in-house test) or 45 (equals 37% with the Cobas test) were found to be positive.

Concordance of the test results:

22 of the samples which were positive in the Cobas test were negative with the in-house Corman-Drosten E gene assay. Two samples were negative in the Cobas test for both gene targets but were positive for the Corman-Drosten E-gene test. 74 of 88 samples were tested negative with both tests (negative agreement 84,1%).

All discordant samples had high Ct-values (35 or higher). The majority of positive samples in the Cobas had CTs of 30-39.

Boutin et al. (Figure 1): from Boutin *et al.* demonstrates high concordance at low Ct with less concordance at high Ct.

Correlation between cycle thresholds (Ct) values obtained with the cobas 8800 SARS-CoV-2 assay for target -1 Orf1 gene and target 2 –E gene (pan-sabercovirus detection) in 279 positive samples for SARS-CoV-2 virus RNA. The dotted line is the 95 % confidence internal of the regression line.

Direct-link to Boutin et al. (Figure 1):
https://pubmed.ncbi.nlm.nih.gov/32927356/#&gid=article-figures&pid=fig-1-uid-0

Re-testing of 20 of the 22 samples that were positive with Cobas but negative at the Charité E-gene, the Abbot system resulted in 8 “detected”, 11 “non detected” and one impossible result.

Re-test of the 11 negative samples with the Abbot test (initially positive with the Cobas test) revealed one positive result in the re-test with the Coabs system and 10 negative results. According to the authors this result was due to limited storage possibility of the samples.

Boutin Discussion:

The authors claim that there is currently no gold standard for the diagnosis of SARS-CoV-2 infection. Limit of detection was now given with 300 SARS-CoV-2 RNA copies per ml sample (was 180 in the Materials section). Despite the difference in the detected samples (negative agreement only 84.1%, so 15.9% difference), the authors conclude that their study demonstrates an excellent agreement between the Cobas Sars-CoV-2 test and the in-house Sarbeco E (Drosten-Corman Test).

Evaluation: the study clearly shows:

- The test system used for PCR defines the type of positive findings (here 68% vs. 80%) with a remarkable high difference (15%) with different tests applied to the same samples.
- The majority of positive samples with both test systems were found at a Ct higher than 30 or even 35.
- Since the findings were not assigned to the symptomatic/asymptomatic/unknown clinical data of patients, no correlation of result against Ct with clinical data is possible.

20. Pfefferle *et al.*

Pfefferle *et al.* used the original Corman E-primer pair and probes, but: ”Both primers were modified with 2’-O-methyl bases in their **penultimate base to prevent formation of primer dimers.“** They did not test the PCR on patient samples, but on *in vitro* transcribed E-Gene RNA of SARS-CoV-2 only. So the authors of this very early publication (submitted Feb 14, 2020) pointed out that the original Drosten/Corman E-gene PCR primers were prone to primer dimers and that the PCR should always be confirmed with a second independent PCR.

The authors note:

"It has to be noted that by its nature as a screening test targeting only a single viral gene, positive results should always be confirmed with an independent PCR as recommended." [42]

b. Summary wet-lab evidence of primer design flaws

In summary, the peer-reviewed literature on the defects of the Corman-Drosten primers is vast. While biases and errors may be understandable due to pandemic time constraints, those due to short-circuited peer review, conflicts of interest and regulatory capture at the WHO, should be condemned once they are identified. There is no way to maintain public trust in the scientific method and publication process when such errors affect millions of people's clinical decisions and livelihoods.

This is no subtle oversight as it is well established in clinical diagnostics that internal controls and Ct correlations with replication competent organisms are a requirement to benchmark any Ct score to biological meaning. Many papers now describe how to properly perform such calibrations with PFU and Ct scores like Jaafar *et al.* [26]. Some even describe more comprehensive methods to understand infectious vs non-infectious patients with careful attention to subgenomic RNA and genomic RNA (Wölfel *et al.* [37] and Liotti *et al.* [27]).

Walker *et al.* [28] even demonstrate that only 72% of the samples produce positive results when 3 gene targets are utilized. 28% of samples only amplify with 1 or 2 of the assays failing, suggestive of degraded and non-infectious RNA due to an amplicon design focus on targeting the highly expressed 3 prime subgenomic RNAs (Figure 24). Assays cutting corners and relying solely on 1 or 2 assays (targeting non-replication competent subgenomic RNA) with no internal controls create erroneous results and quarantines an excessive number of non-infectious people.

Figure 26: Table reproduced from Walker *et al.* demonstrating the number of positive tests where 1,2 and 3 genes amplify.

Table 1 Genes detected in positive swabs

	All positives (N=1892)		First positive per participant (N=1516)	
Number of genes detected	n (%)	Median CT* (IQR) [range]	n (%)	Median CT* (IQR) [range]
1	345 (18%)	33.6 (32.3-34.6) [12.7-37.6]	307 (20%)	33.7 (32.5-34.7) [12.7-37.3]
2	185 (10%)	31.5 (29.8-32.8) [10.3-36.3]	138 (9%)	31.5 (29.6-33.0) [10.3-36.3]
3	1362 (72%)	22.8 (18.2-27.4) [10.5-34.2]	1071 (71%)	21.8 (17.7-27.0) [10.5-33.8]
Genes detected				
N only	243 (13%)	33.7 (32.5-34.7) [29.0-37.6]	213 (14%)	33.8 (32.6-34.7) [29.0-37.1]
ORF1ab only	83 (4%)	32.7 (31.9-33.8) [24.0-35.7]	75 (5%)	33.0 (31.9-33.9) [24.0-35.7]
S only**	19 (1%)	35.0 (34.3-36.1) [12.7-37.3]	19 (1%)	35.0 (34.3-36.1) [12.7-37.3]
N+ORF1ab	158 (8%)	31.3 (29.8-32.6) [10.3-36.3]	113 (7%)	31.2 (29.6-32.8) [10.3-36.3]
S+ORF1ab	9 (0.5%)	28.9 (26.1-31.0) [16.2-34.7]	8 (0.5%)	28.8 (24.5-32.1) [16.2-34.7]
N+S	18 (1%)	32.8 (32.3-33.1) [28.2-35.2]	17 (1%)	32.8 (32.3-33.1) [28.2-35.2]
N+S+ORF1ab	1362 (72%)	22.8 (18.2-27.4) [10.5-34.2]	1071 (71%)	21.8 (17.7-27.0) [10.5-33.8]

* taking the mean CT per positive swab across positive gene targets (Spearman rho=0.99 for each pair of genes, p<0.0001)
** through mid-May only: after this samples positive for the S gene only were not called positive overall.
Note: comparing first vs subsequent positives per participant, exact p<0.0001 for both number of genes detected and specific genes detected.

Liotti *et al.* [27] demonstrate qPCR positivity can last as long as 77 days (48.6 mean) past symptom development but only observe 7-10 days of infectiousness (n=176) when benchmarking the Ct values against cell culture. Increasing the sample size of a study like Liotti *et al*. is likely to find cases that extend the long tail of qPCR positivity post-recovery and post-infectiousness. Liotti *et al.* implies the vast majority of qPCR positive samples will be non-infectious patients. They describe a mean of 48.6 days of qPCR positive. They also describe 7-10 days of infectiousness. This produces a range of non-infectious qPCR positive to infectious qPCR positive ratio of 4.86:1 to 11:1. This is an alarming rate of quarantine for non-infectious patients. To quarantine a patient, you must have evidence of existing infectiousness, not RNA from a past infection. The Corman-Drosten manuscript ignores this medical ethics question whilst also compromising the accelerated peer-review process by a gross failure to disclose financial conflicts of interest.

The authors' premature escalation of their work to the WHO prior to peer review is alarming. The lab testing revenue and therefore conflicts of interest of various authors were not properly disclosed in the initial Eurosurveillance peer review. Had the journal been aware of the conflicts they may have placed more scrutiny on the review.

Likewise, we have not seen the authors exhibit the same urgency in updating the WHO regarding the reported false positives from the hastily reviewed Corman-Drosten paper. This raises important questions regarding the lab testing conflicts of interest of various authors.

Increased qPCR positivity amplifies testing revenue through follow-on track-and-trace testing revenue. This places public health and citizen freedom in direct conflict with heavily funded testing labs who clearly have financial interests in higher test positivity.

Section 2:

B. Meta-data Analysis on EuroSurveillance.org (peer review timeframes)

Additional work was provided profiling the peer-review timeframes at Eurosurveillance by Wouter Aukema, who has over 30 years of experience in processing and analysing data for governments and corporations world-wide and develops data analysis solutions for Fortune 100 companies. His publication at Defcon (20 years ago) caused headlines worldwide as it identified significant software virus vulnerabilities to Lotus Notes [29].

This analysis by Wouter Aukema provides additional evidence of the exceptional short review time for a manuscript that, at the time, didn't fully disclose the authors' conflicts of interests. This puts the journal in a very compromised position as it may have been scrutinised more had the conflicts been disclosed during the rushed review. Instead these conflicts were brought to light after the rushed review and publication.

The goal is to understand how much time it typically takes for research papers to get reviewed and accepted by eurosurveillance.org. [29], (Figure 27);

The reason for this assessment is to provide clarity around discussions of a specific research paper that was reviewed and accepted in a single day. Some scientists think it is impossible to Peer-Review research within a single day. Other scientists claim the paper went through the much quicker- Rapid Review procedure, as outlined on the journal's web site.

To assess commonality in the review and acceptance process at eurosurveillance.org, the author collected and analysed meta-data for all 1,595 publications since 01-Jan-2015. Earlier this week, the author shared the initial findings of this assessment in a Twitter post.

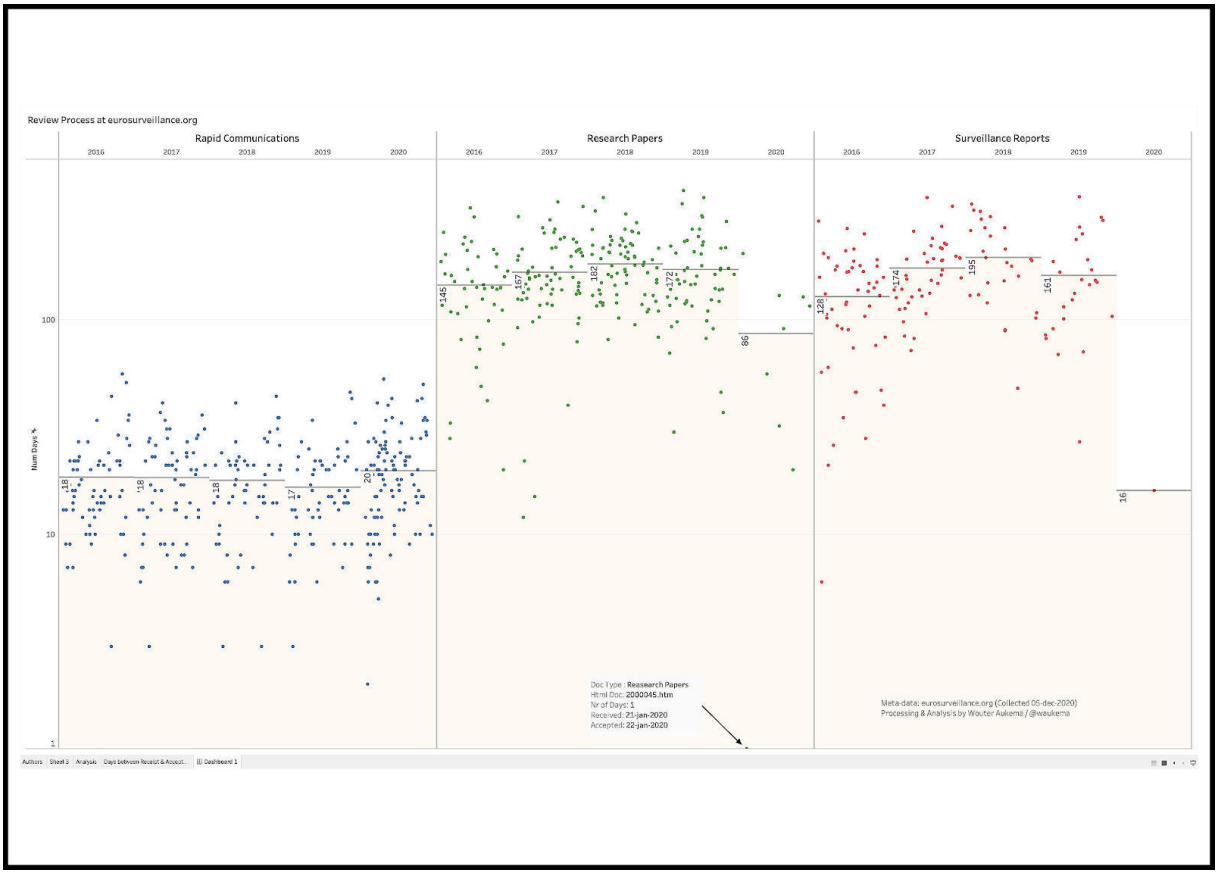
This six-page document aims to make these findings reproducible and verifiable by offering step by step instructions.

Summary of Findings:

- *Of the 17 types of articles published since 2015, three types occur most frequently: Rapid Communication (385), Research (312) and Surveillance (193).*

- *The average number of days between Acceptance and Reception of Research type articles is 172 (2019) and 97 (2020).*
- *In line with the Editorial Policy for Authors, the category 'Rapid Communication' publications appear to be reviewed and accepted more quickly (18 days average) than type 'Research' and 'Surveillance.'*
- *Except for this one Research article (on 22-jan-2020), no other article has ever been reviewed and accepted within a single day since 2015.*

Figure 27: Dot plot of peer review timelines for manuscripts published at Eurosurveillance since 2015. The Corman-Drosten paper is an extreme outlier.



The corresponding author (in this case Christian Drosten) had to fill out a section called “Agreement with authors” at the Eurosurveillance Submission portal, a mandatory requirement and document for successful submission. Christian Drosten had to confirm that there were no conflicts of interests. We can clearly conclude that he was not honest while filling out the form back in January 2020. Six months later into the pandemic an Update was added for Marco Kaiser under the section “conflicts of interests”, who is senior researcher at

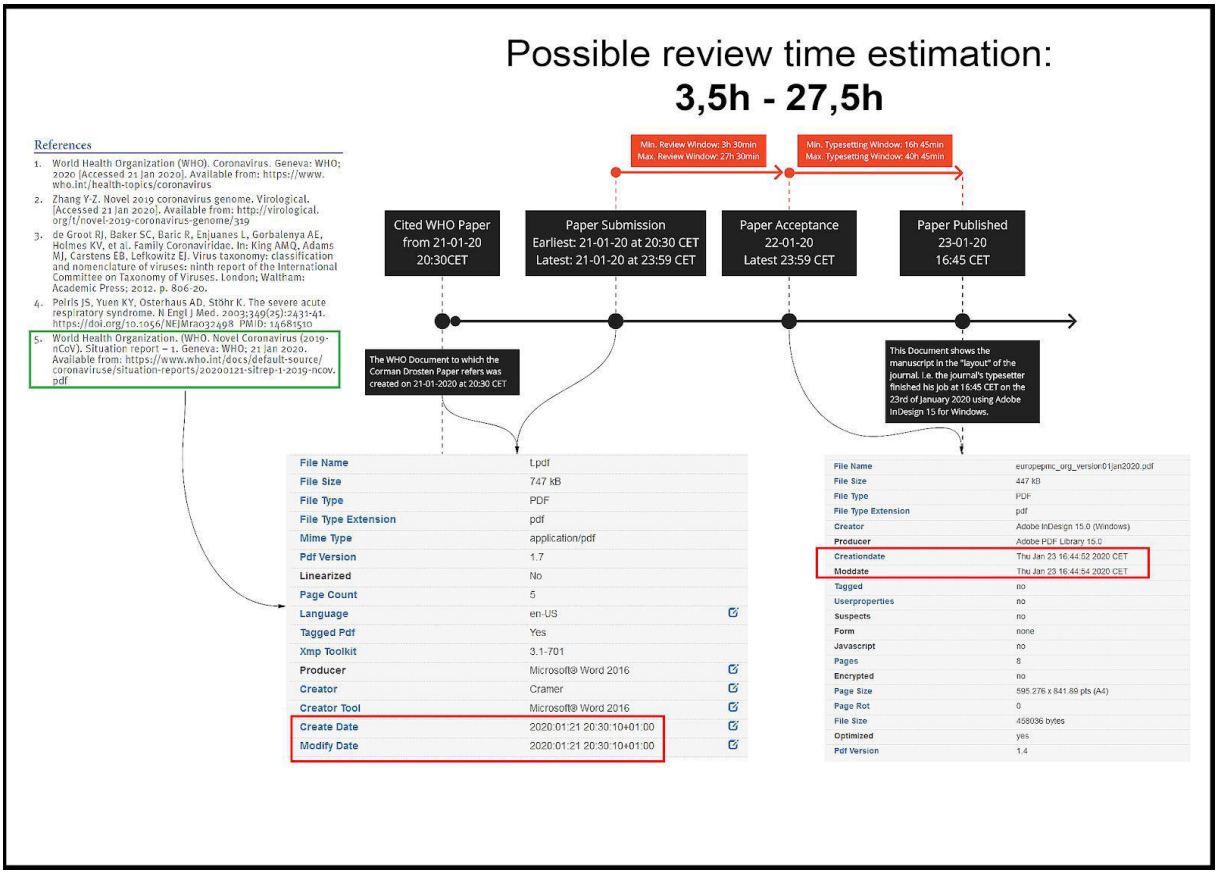
GenExpress and serves as scientific advisor for Tib-Molbiol. Given the unbelievably short review time, we have to further conclude:

- The editor in charge found experts that are willing to review within hours.
- All experts immediately reviewed the manuscript and declared it as perfect, as it is.
- The editor immediately handled the review reports.

Nevertheless, after acceptance the paper still needs to be sent to a typesetter, even though it had immediately received the “Accept” status without any major or minor revisions.

The timeline of the Corman-Drosten Peer Review demonstrates digital timestamps on documents sent to the WHO at 20:30 CET on Jan 21-2020. The paper submitted to Eurosurveillance on the same day references the WHO document and is assumed to have been submitted after 20:30 CET as it's impossible to reference a WHO document unless the WHO document was submitted first. This leaves 3.5hrs to 27.5 hrs for review as the paper was accepted the next day on Jan 22, 2020. Given the late evening submission, reviewers would have to be recruited off-hours, agree to review the paper and complete the review mostly outside of business hours. (Figure 28)

Figure 28: Possible review time estimation Corman Drosten et al.



C. Missing positive controls for PCR test validation

This chapter further investigates the positive controls referenced in Muenchhoff *et al*, Mautheeussen *et al.* and Wolf *et al.*

The positive controls used to prepare the RNA dilution series as the basis for the Corman RT-PCR-testing were described as a sample deriving from a five-year-old child with COVID-19. As source, Wolf *et al.* is cited. The methods section states:

"*Nasopharyngeal swabs were used for virus culture in a biosafety level 3 laboratory on Vero cells.*" [6]

The results section of the Wolf *et al*. paper which is referenced in the Muenchhoff *et al*. paper further concludes:

"*She did not develop any respiratory symptoms but tested PCR-positive again in nasal and pharyngeal swabs on 3rd February when infectious viruses could be grown from swab material."* [6]

According to Wolf *et al.,* a pathogen was isolated and cultured from the patient. Further, for her two-year old brother, they noticed:

"As with his sister, the infectious virus was easily grown from the nasopharyngeal swab material on 3rd and 4th February." [6]

Following these statements there should have been two virus isolates available for the Muenchhoff *et al.* study (submitted 28th May 2020), but they didn't characterize the RNA isolated from the samples. The Matheeussen *et al.* publication [33] (submitted two weeks later), claimed that SARS-CoV-2 isolates are used as a source for the positive control RNA.

Neither the Wolf *et al.* publication, nor the Muenchhoff *et al.* or Matheeussen *et al.* describe how the virus isolates / RNA used in the assay validation is characterized. There is no data available concerning sequence validation of these targets and no information about the virus in general.

Wolf *et al.* and Muenchhoff *et al.* list the Institute for Virology in Munich as the main research-hub / institution & correspondence. Christian Drosten is co-author of the Muenchhoff *et al.* & Mautheussen *et al.* publications. Victor Corman is the second author of the latter paper. The audit trail for the "true positive" controls used for the basis of the test is thus incomplete. This makes it impossible for labs to directly replicate the work.

In a recent Lancet publication Surkova *et al*.[46] it is stated:

"RT-PCR tests to detect severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA are the operational gold standard for detecting COVID-19 disease in clinical practice.
[...], but no single gold standard assay exists." [46]

In other words, the sensitivity and specificity of PCR are determined with the PCR test itself as "operational gold standard". PCR tests should be calibrated to replication competent organisms. Use of PCR to validate PCR is circular reasoning.

Surkova *et al.* references a British Medical Journal article, Watson *et al*., and there we can find following further conclusions:

"*No test gives a 100% accurate result; tests need to be evaluated to determine their sensitivity and specificity, ideally by comparison with a "gold standard." The lack of such a clear-cut "gold-standard" for covid-19 testing makes evaluation of test accuracy challenging.*
A systematic review of the accuracy of covid-19 tests reported false negative rates of between 2% and 29% (equating to sensitivity of 71-98%), based on negative RT-PCR tests which were positive on repeat testing. The use of repeat RT-PCR testing as gold standard is likely to underestimate the true rate of false negatives, as not all patients in the included studies received repeat testing and those with clinically diagnosed covid-19 were not considered as actually having covid-19." [46]

D. *In silico* Analysis, Primer homology to human DNA

We have performed additional analysis to address concerns voiced regarding the Charité primers and their homology to human DNA.

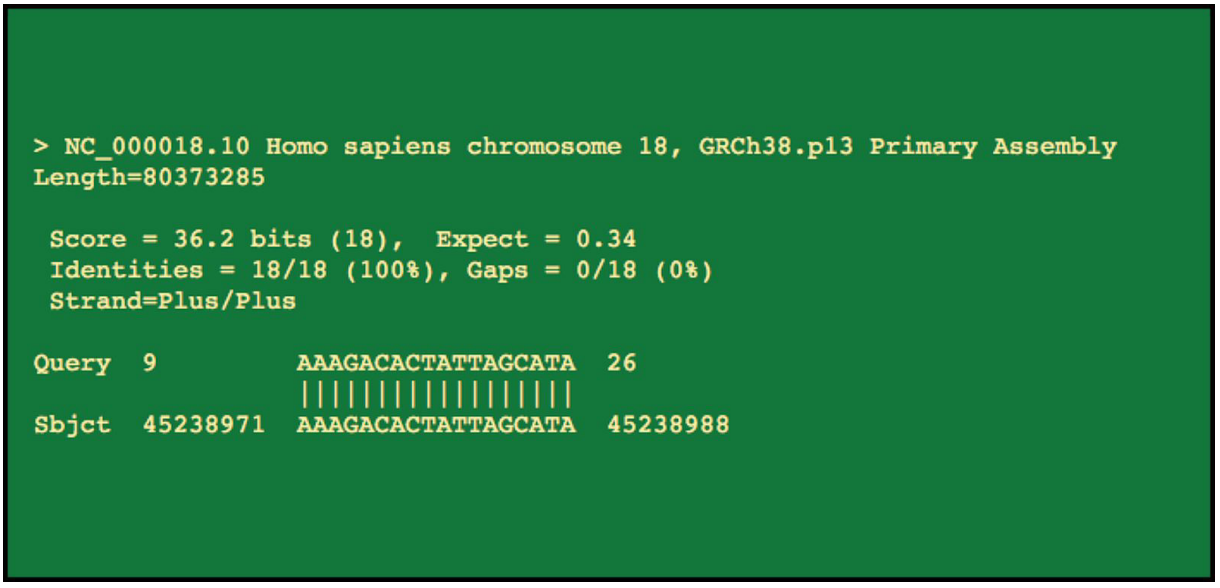
We have included a BLAST analysis of the Charité primers against the Human Genome (GRCh38.p13). There are several significant homologies but none that have both primer and probes in close proximity. While these off-target homologies are not catastrophic for assay performance, they do demonstrate the lack of *in silico* analysis done prior to publication and they may play a role in the in-vitro synthesis of more diverse 3 prime ends of primers during the cold (55C) reverse transcription step of RT-qPCR. The BLAST output file is available for download in the references section [30]. With the shortage of RNA purification kits in 2020, many labs are using modified purification protocols that omit the DNAse step thus leaving human DNA as a viable target of primers (Figure 28) [32].

Wozniak et al. describe a more automatable and streamlined RNA preparation for SARs-CoV-2 qPCR. They omit the DNase step to reduce consumables and notice it benefits their internal control signal. The authors conclude:

"DNase treatment is not necessary because SARS-CoV-2 detection is not altered in the presence of DNA. In fact, residual DNA may serve as the template for RNase P gene amplification."

Figure 29 shows the 18bp 3 prime homology found in the RdRp Reverse primer to human chromosome 18.

Figure 29: BLAST alignment using blastdb -task blastn-short -query Corman_Primers.fa -db GRCh38.p13.fna. Query is the RdRp Reverse primer and Sbjct = Human Genome reference genome GRCh38.p13 Primary Assembly in NCBI.



E. Further Discussion - The Consequences of False Positives / False Negatives

We further conclude that the origin of the problem is not solely technical in nature but also not fit for the intended clinical purpose in the Corman Drosten-paper.

We aimed to develop and deploy robust diagnostic methodology for use in public health laboratory settings without having virus material available. [44]

This misguided aim is already discussed in the main review report Pieter Borger *et al*. [4] in great detail, nevertheless we see the need to re-emphasize the misguided premise at this point and to extend our critique on population mass-testing through the means of RT-qPCR.

Even if the RT-qPCR test was optimal and had theoretically sensitivity and specificity of 100%, it is medical malpractice to use RT-qPCR and other rapid tests outside the need for specific antiviral therapy in symptomatic or severely ill hospitalised patients. Interpreting

positive tests as 'medical cases' without consideration of internal controls and viral Ct with clinical context, nor consideration of other viruses or diseases that cause similar symptoms as COVID-19, enables politicians to practice medicine on entire populations. This lack of clinical integration has led to problems in the past.

Blind faith in a quick RT-qPCR-test has created a pseudo-epidemic described in this New York Times article in 2007 [34]:

"I had a feeling at the time that this gave us a shadow of a hint of what it might be like during a pandemic flu epidemic.
[...]
Yet, epidemiologists say, one of the most troubling aspects of the pseudo-epidemic is that all the decisions seemed so sensible at the time."

Even Christian Drosten admitted himself in a German Article in 2014 the very problem of RT-qPCR tests in a pandemic or epidemic scenario:

"The method is so sensitive that it can detect a single genetic molecule of the virus. If, for example, such a pathogen flies over the nasal mucous membrane of a nurse for a day without them becoming ill or noticing anything, then it is suddenly a MERS case. Where previously terminally ill were reported, now suddenly mild cases and people who are actually very healthy are included in the reporting statistics. This could also explain the explosion in the number of cases in Saudi Arabia." [45]

Furthermore, the WHO falsely claims in an official document:

"In areas where COVID-19 virus is widely spread a simpler algorithm might be adopted in which, for example, screening by rRT-PCR of a single discriminatory target is considered sufficient." [48]

A single confirmatory gene assay can never be sufficient enough for accurate testing-results, especially not in a mass-testing scenario. [42]

The PCR testing with the E-gene (Corman-Drosten *et al.*) is also used in single-gene PCR tests in the EU and has been demonstrated to be unspecific for the detection of SARS-CoV-2 [49].

"A high amount of specificity means, that the test is able to detect SARS-CoV-2 infections, only. In contrast, PCR tests with a rather lower specificity might pick up all kinds of other Corona viruses. The lower the specificity, the lower the ability to prove the infection by a specific virus." [47]

This is an important point to underscore. According to Corman *et al*. they describe their RdRp gene as having low specificity yet this is a confirmatory assay that has many design flaws and documented deficiencies in the literature.

"Detection of these phylogenetic outliers within the SARS-related CoV clade suggests that all Asian viruses are likely to be detected." [44]

The E-gene also has documented deficiencies and the test has no internal controls or calibration to replication competent organisms or PFUs. The genbank accession numbers in NCBI do not contain any E gene sequences to demonstrate the assay is functional.

The Corman Drosten-protocol results can not be reproduced.

The consequences of false-positives are further discussed in an article by Howard Steen & Saji Homeed [35] and in an article by Michael Yeadon, titled *The PCR False Positive Pseudo-Epidemic* [36].

Section 3: References

[1] Muenchhoff Maximilian, Mairhofer Helga, Nitschko Hans, Grzimek-Koschewa Natascha, Hoffmann Dieter, Berger Annemarie, Rabenau Holger, Widera Marek, Ackermann Nikolaus, Konrad Regina, Zange Sabine, Graf Alexander, Krebs Stefan, Blum Helmut, Sing Andreas, Liebl Bernhard, Wölfel Roman, Ciesek Sandra, Drosten Christian, Protzer Ulrike, Boehm Stephan, Keppler Oliver T. Multicentre comparison of quantitative PCR-based assays to detect SARS-CoV-2, Germany, March 2020. Euro Surveill. 2020;25(24):pii=2001057. https://doi.org/10.2807/1560-7917.ES.2020.25.24.2001057

[2] Official recommendation of the Corman-Drosten protocol & manuscript by the WHO,published on January 13th 2020 as version 1.0 of the document: https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287a1b17c111902.pdf; Archive: https://bit.ly/3m3jXVH

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

[4] Borger, Pieter, Malhotra, Rajesh Kumar, Yeadon, Michael, Craig, Clare, McKernan, Kevin, Steger, Klaus, ... Kämmer, Ulrike. (2020). External peer review of the RTPCR test to detect SARS-CoV-2 reveals 10 major scientific flaws at the molecular and methodological level: consequences for false positive results. http://doi.org/10.5281/zenodo.4298004
https://zenodo.org/record/4298004#.X8T4b7cxmUk

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

[6] Wolf, G. K., Glueck, T., Huebner, J., Muenchhoff, M., Hoffmann, D., French, L. E., Keppler, O. T., & Protzer, U. (2020). Clinical and Epidemiological Features of a Family Cluster of Symptomatic and Asymptomatic Severe Acute Respiratory Syndrome Coronavirus 2 Infection. *Journal of the Pediatric Infectious Diseases Society*, *9*(3), 362–365. https://doi.org/10.1093/jpids/piaa060
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7313851/pdf/piaa060.pdf

[7] Yujin Jung, Gun-Soo Park, Jun Hye Moon, Keunbon Ku, Seung-Hwa Beak, Chang-Seop Lee, Seil Kim, Edmond Changkyun Park, Daeui Park, Jong-Hwan Lee, Cheol Woo Byeon, Joong Jin Lee, Jin-Soo Maeng, Seong-Jun Kim, Seung Il Kim, Bum-Tae Kim, Min Jun Lee, and Hong Gi Kim. Comparative Analysis of Primer–Probe Sets for RT-qPCR of COVID-19 Causative Virus (SARS-CoV-2). ACS Infectious Diseases 2020 (9), 2513-2523. DOI: 10.1021/acsinfecdis.0c00464.
https://pubs.acs.org/doi/10.1021/acsinfecdis.0c00464

[8] Etievant, S., Bal, A., Escuret, V., Brengel-Pesce, K., Bouscambert, M., Cheynet, V., Generenaz, L., Oriol, G., Destras, G., Billaud, G., Josset, L., Frobert, E., Morfin, F., & Gaymard, A. (2020). Performance Assessment of SARS-CoV-2 PCR Assays Developed by WHO Referral Laboratories. *Journal of clinical medicine*, *9*(6), 1871. https://doi.org/10.3390/jcm9061871
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7355678/

[9] Klement, R.J., Bandyopadhyay, P.S. The Epistemology of a Positive SARS-CoV-2 Test. *Acta Biotheor* (2020).
https://doi.org/10.1007/s10441-020-09393-w
https://link.springer.com/article/10.1007/s10441-020-09393-w

[10] Gand M, Vanneste K, Thomas I, Van Gucht S, Capron A, Herman P, Roosens NHC, De Keersmaecker SCJ. Use of Whole Genome Sequencing Data for a First in Silico Specificity Evaluation of the RT-qPCR Assays Used for SARS-CoV-2 Detection. *International Journal of Molecular Sciences*. 2020; 21(15):5585. https://www.mdpi.com/1422-0067/21/15/5585

[11] Charité's response to Partsch & Partner Attorneys Christoph J. Partsch inquiry / questionnaire:
http://schlussjetzt.org/Antwortschreiben%20Charite%20auf%20Pressefragen%20Jens%20Wernicke%20vom%2018.06.2020.pdf

[12] Konrad Regina, Eberle Ute, Dangel Alexandra, Treis Bianca, Berger Anja, Bengs Katja, Fingerle Volker, Liebl Bernhard, Ackermann Nikolaus, Sing Andreas. Rapid establishment of laboratory diagnostics for the novel coronavirus SARS-CoV-2 in Bavaria, Germany, February 2020. Euro Surveill. 2020;25(9):pii=2000173. https://doi.org/10.2807/1560-7917.ES.2020.25.9.2000173
https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.9.2000173

[13] Sethuraman N, Jeremiah SS, Ryo A. Interpreting Diagnostic Tests for SARS-CoV-2. *JAMA.* 2020;323(22):2249–2251. doi:10.1001/jama.2020.8259
https://jamanetwork.com/journals/jama/article-abstract/2765837

[14] Vogels, C.B.F., Brito, A.F., Wyllie, A.L. *et al.* Analytical sensitivity and efficiency comparisons of SARS-CoV-2 RT–qPCR primer–probe sets. *Nat Microbiol* 5, 1299–1305 (2020). https://doi.org/10.1038/s41564-020-0761-6
https://www.nature.com/articles/s41564-020-0761-6

[15] Kevin S. Kuchinski, Agatha N. Jassem, Natalie A. Prystajecky, Assessing oligonucleotide designs from early lab developed PCR diagnostic tests for SARS-CoV-2 using the PCR_strainer pipeline, Journal of Clinical Virology, Volume 131, 2020, 104581, ISSN 1386-6532,
https://doi.org/10.1016/j.jcv.2020.10458 https://www.sciencedirect.com/science/article/pii/S1386653220303231

[16] Pillonel, T., Scherz, V., Jaton, K., Greub, G., & Bertelli, C. (2020). Letter to the editor: SARS-CoV-2 detection by real-time RT-PCR. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin*, *25*(21), 2000880.
https://doi.org/10.2807/1560-7917.ES.2020.25.21.2000880
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7268274/

[17] Jeremy Ratcliff, Dung Nguyen, Monique Andersson, Peter Simmonds, bioRxiv 2020.06.24.168013; Evaluation of Different PCR Assay Formats for Sensitive and Specific Detection of SARS-CoV-2 RNA, doi: https://doi.org/10.1101/2020.06.24.168013
https://www.biorxiv.org/content/10.1101/2020.06.24.168013v2.full.pdf

[18] Lauren Hubert Jaeger, Thiago César Nascimento, Fabíola Dutra Rocha, Fernanda Maria Pinto Vilela, Ana Paula do Nascimento Duque, Lívia Mara Silva, Lorena Rodrigues Riani, João Paulo Moreira, Jéssica Mara de Assis Chagas, Thamiris Vilela Pereira, Carmen Gomide Pinto Perches, Aripuana Sakurada Aranha Watanabe, Lyderson Facio Viccini, Marcelo Silva Silvério, José Otávio do Amaral Corrêa, Olavo dos Santos Pereira-Junior, Frederico Pittella, Adjusting RT-qPCR conditions to avoid unspecific amplification in SARS-CoV-2 diagnosis, International Journal of Infectious Diseases, Volume 102, 2021, Pages 437-439, ISSN 1201-9712, https://doi.org/10.1016/j.ijid.2020.10.079. https://www.sciencedirect.com/science/article/pii/S1201971220322839

[19] Khan KA, Cheung P. 2020 Presence of mismatches between diagnostic PCR assays and coronavirus SARS-CoV-2 genome. R. Soc. Open Sci. 7: 200636. http://dx.doi.org/10.1098/rsos.200636
https://royalsocietypublishing.org/doi/pdf/10.1098/rsos.200636

[20] Opota, Onya, Brouillet, René, Greub, Gilbert, Jaton, Katia, Comparison of SARS-CoV-2 RT-PCR on a high-throughput molecular diagnostic platform and the cobas SARS-CoV-2 test for the diagnostic of COVID-19 on various clinical samples, Pathogens and Disease, Pathog Dis, 2020, DOI: 10.1093/femspd/ftaa061
https://academic.oup.com/femspd/article/78/8/ftaa061/5919476?login=true

[21] Gustavo Barcelos Barra, Ticiane Henriques Santa Rita, Pedro Góes Mesquita, Rafael Henriques Jácomo, Lídia Freire Abdalla Nery, Analytical sensibility and specificity of two RT-qPCR protocols for SARS-CoV-2 detection performed in an automated workflow, https://doi.org/10.1101/2020.03.07.20032326
https://www.medrxiv.org/content/10.1101/2020.03.07.20032326v1.full.pdf

[22] Santos, Renan da Silva, Bret, Raissa Souza Caminha, Moreira, Ana Cristina de Oliveira Monteiro, Campos, Adriana Rolim, Silva, Angelo Roncalli Alves e, Lima, Danielle Malta, & Tavares, Kaio Cesar Simiano. (2020). In silico analysis of mismatches in RT-qPCR assays of 177 SARS-CoV-2 sequences from Brazil. *Revista da Sociedade Brasileira de Medicina Tropical*, *53*, e20200657. Epub November 25, 2020.https://doi.org/10.1590/0037-8682-0657-2020
https://www.scielo.br/scielo.php?pid=S0037-86822020000100668&script=sci_arttext

[23] Anantharajah et al., Raphael Helaers, Jean-Philippe Defour, Nathalie Olive, Florence Kabera, Luc Croonen, Françoise Deldime, Jean-Luc Vaerman, Cindy Barbée, Monique Bodéus, Anais Scohy, Alexia Verroken, Hector Rodriguez-Villalobos, Benoît Kabamba-Mukadi, How to choose the right real-time RT-PCR primer sets for the SARS-CoV-2 genome detection?, 2020, DOI: https://doi.org/10.21203/rs.3.rs-36512/v1
https://assets.researchsquare.com/files/rs-36512/v1/d7f58eb9-bc63-44f3-afe6-6fc01a077704.pdf

[24] Arun K. Nalla, Amanda M. Casto, Meei-Li W. Huang, Garrett A. Perchetti, Reigran Sampoleo, Lasata Shrestha, Yulun Wei, Haiying Zhu, Keith R. Jerome, Alexander L. Greninger, Comparative Performance of SARS-CoV-2 Detection Assays Using Seven Different Primer-Probe Sets and One Assay Kit, Journal of Clinical Microbiology May 2020, 58 (6) e00557-20; **DOI:** 10.1128/JCM.00557-20
https://jcm.asm.org/content/58/6/e00557-20

[25] Elias Dahdouh, Fernando Lázaro-Perona, María Pilar Romero-Gómez, Jesús Mingorance, Julio García-Rodriguez, Ct values from SARS-CoV-2 diagnostic PCR assays should not be used as direct estimates of viral load, 2020, DOI:https://doi.org/10.1016/j.jinf.2020.10.017
https://www.journalofinfection.com/article/S0163-4453(20)30675-7/fulltext

[26] Rita Jaafar, Sarah Aherfi, Nathalie Wurtz, Clio Grimaldier, Thuan Van Hoang, Philippe Colson, Didier Raoult, Bernard La Scola, Correlation Between 3790 Quantitative Polymerase Chain Reaction–Positives Samples and Positive Cell Cultures, Including 1941 Severe Acute Respiratory Syndrome Coronavirus 2 Isolates, *Clinical Infectious Diseases*, , ciaa1491, https://doi.org/10.1093/cid/ciaa1491
https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciaa1491/5912603

[27] Liotti FM, Menchinelli G, Marchetti S, et al. Assessment of SARS-CoV-2 RNA Test Results Among Patients Who Recovered From COVID-19 With Prior Negative Results. *JAMA Intern Med.* Published online November 12, 2020. doi:10.1001/jamainternmed.2020.7570
https://jamanetwork.com/journals/jamainternalmedicine/fullarticle/2773053

[28] A. Sarah Walker, Emma Pritchard, Thomas House, Julie V Robotham, Paul J Birrell, Iain Bell, John I Bell, John N Newton, Jeremy Farrar, Ian Diamond, Ruth Studley, Jodie Hay, Karina-Doris Vihta, Tim Peto, Nicole Stoesser, Philippa C. Matthews, David W. Eyre, Koen B. Pouwels, the COVID-19 Infection Survey team, Viral load in community SARS-CoV-2 cases varies widely and temporally, 2020, https://doi.org/10.1101/2020.10.25.20219048
https://www.medrxiv.org/content/10.1101/2020.10.25.20219048v1.full.pdf

[29] Wouter Aukema, Meta-data Analysis of eurosurveillance.org

Blog Entry Archive Link: https://archive.is/QZm87,
Blog Entry (Web Archive Timestamp 8.12.2020): http://bit.ly/3mS1YAS,
PDF: http://bit.ly/3aCj3wm

[30] BLAST output file by Kevin McKernan:
Supplementary Material Addendum Review Report Corman Drosten et al.

[31] Corman Victor M, Drosten Christian. Authors' response: SARS-CoV-2 detection by real-time RT-PCR. Euro Surveill. 2020;25(21):pii=2001035. https://doi.org/10.2807/1560-7917.ES.2020.25.21.2001035
https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.21.2001035#r1

[32] Wozniak, A., Cerda, A., Ibarra-Henríquez, C. *et al.* A simple RNA preparation method for SARS-CoV-2 detection by RT-qPCR. *Sci Rep* 10, 16608 (2020). https://doi.org/10.1038/s41598-020-73616-w
https://www.nature.com/articles/s41598-020-73616-w

[33] Matheeussen Veerle, Corman Victor M, Donoso Mantke Oliver, McCulloch Elaine, Lammens Christine, Goossens Herman, Niemeyer Daniela, Wallace Paul S, Klapper Paul, Niesters Hubert GM, Drosten Christian, Ieven Margareta, on behalf of the RECOVER project and collaborating networks. International external quality assessment for SARS-CoV-2 molecular detection and survey on clinical laboratory preparedness during the COVID-19 pandemic, April/May 2020. Euro Surveill. 2020;25(27):pii=2001223.
https://doi.org/10.2807/1560-7917.ES.2020.25.27.2001223
https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.27.2001223

[34] Gina Kolata, Faith in Quick Test Leads to Epidemic That Wasn't, New York Times, 2007,
Archive Link: https://archive.is/ugiWQ

[35] Howard Steen & Saji Homeed - The consequences of false positives:
https://cormandrostenreview.com/false-positives-consequences/

[36] Michael Yeadon, The PCR False Positive Pseudo-Epidemic:
https://lockdownsceptics.org/the-pcr-false-positive-pseudo-epidemic/

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

[38] Lightmix PCR Roché / Tib Molbiol:
https://www.tib-molbiol.com/company/news.html?id=142&img=185

[39] Kevin Poulsen, SecurityFocus 2000-07-29, Hackers take 'Notes' in Vegas
https://www.securityfocus.com/news/66

[40] Mario Poljak, Miša Korva, Nataša Knap Gašper, Kristina Fujs Komloš, Martin Sagadin, Tina Uršič, Tatjana Avšič Županc, Miroslav Petrovec, Clinical evaluation of the cobas SARS-CoV-2 test and a diagnostic platform switch during 48 hours in the midst of the COVID-19 pandemic, Journal of Clinical Microbiology Apr 2020, JCM.00599-20; **DOI:** 10.1128/JCM.00599-20
https://jcm.asm.org/content/early/2020/04/09/JCM.00599-20/article-info?versioned=true

[41] Boutin CA, Grandjean-Lapierre S, Gagnon S, Labbé AC, Charest H, Roger M, Coutlée F. Comparison of SARS-CoV-2 detection from combined nasopharyngeal/oropharyngeal swab samples by a laboratory-developed real-time RT-PCR test and the Roche SARS-CoV-2 assay on a cobas 8800 instrument. J Clin Virol. 2020 Nov;132:104615. doi: 10.1016/j.jcv.2020.104615. Epub 2020 Sep 4. PMID: 32927356; PMCID: PMC7472968. https://pubmed.ncbi.nlm.nih.gov/32927356/

[42] Pfefferle Susanne, Reucher Svenja, Nörz Dominic, Lütgehetmann Marc. Evaluation of a quantitative RT-PCR assay for the detection of the emerging coronavirus SARS-CoV-2 using a high throughput system. Euro Surveill. 2020;25(9):pii=2000152.
https://doi.org/10.2807/1560-7917.ES.2020.25.9.2000152
https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.9.2000152

[43] Kirchdoerfer R.N., Ward A.B. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 cofactors. Nat. Commun. 2019;10(1):2342
https://pubmed.ncbi.nlm.nih.gov/31138817/

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

[45] Interview 2014, with Wirtschaftswoche Magazin, Christian Drosten
https://www.wiwo.de/technologie/forschung/virologe-drosten-im-gespraech-2014-die-who-kann-nur-empfehlungen-aussprechen/9903228-2.html

[46]Surkova et al.False-positive COVID-19 results: hidden problems and costs
https://www.thelancet.com/action/showPdf?pii=S2213-2600%2820%2930453-7

[47] Watson Jessica, Whiting Penny F, Brush John E. Interpreting a covid-19 test result BMJ 2020; 369 :m1808
https://www.bmj.com/content/369/bmj.m1808

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

[49] Cyril Chik-Yan Yip, Siddharth Sridhar, Andrew Kim-Wai Cheng, Kit-Hang Leung, Garnet Kwan-Yue Choi, Jonathan Hon-Kwan Chen, Rosana Wing-Shan Poon, Kwok-Hung Chan, Alan Ka-Lun Wu, Helen Shuk-Ying Chan, Sandy Ka-Yee Chau, Tom Wai-Hin Chung, Kelvin Kai-Wang To, Owen Tak-Yin Tsang, Ivan Fan-Ngai Hung, Vincent Chi-Chung Cheng, Kwok- Yung Yuen, Jasper Fuk-Woo Chan, Evaluation of the commercially available LightMix® Modular E-gene kit using clinical and proficiency testing specimens for SARS-CoV-2 detection, Journal of Clinical Virology, Volume 129, 2020, 104476,
ISSN 1386-6532, https://doi.org/10.1016/j.jcv.2020.104476.
https://www.sciencedirect.com/science/article/abs/pii/S1386653220302183

[50] Borger, Pieter, Malhotra, Rajesh Kumar, Yeadon, Michael, Craig, Clare, McKernan, Kevin, Steger, Klaus, ... Kämmer, Ulrike. (2020). External peer review of the RTPCR test to detect SARS-CoV-2 reveals 10 major scientific flaws at the molecular and methodological level: consequences for false positive results. http://doi.org/10.5281/zenodo.4298004
https://zenodo.org/record/4298004#.X8T4b7cxmUk

[51] Borger et al.
https://www.researchgate.net/publication/346483715_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

[52] Reusken, C., Broberg, E. K., Haagmans, B., Meijer, A., Corman, V. M., Papa, A., Charrel, R., Drosten, C., Koopmans, M., Leitmeyer, K., & On Behalf Of Evd-LabNet And Erli-Net (2020). Laboratory readiness and response for novel coronavirus (2019-nCoV) in expert laboratories in 30 EU/EEA countries, January 2020. *Euro surveillance : bulletin Europeen sur les maladies transmissibles = European communicable disease bulletin*, *25*(6), 2000082. https://doi.org/10.2807/1560-7917.ES.2020.25.6.2000082
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7029448/

[53] Graham et al. SARS coronavirus replicase proteins in pathogenesis, doi: 10.1016/j.virusres.2007.02.017
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2637536/

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