Fwd: Freedom of information - TL1S - FOI 11035 - Final response

[redacted] Tue, Jul 27, 2021 at 3:49 AM

To: christinem@fluoridefreepeel.ca

- TL1S - FOI 11035 - Final response

Good morning,

The Trust has now completed collation of data to support a response to your recent Freedom of Information requests for Pennine Acute NHS Trust and Salford Royal NHS Foundation Trust.

Details requested have been attached to this email.

As part of the disclosure log, all responses to Freedom of Information requests are posted on the Trust's website. Please click on the link below to view the response to your request.

https://www.srft.nhs.uk/about-us/freedom-of-information/randr/?entryid34=218053&q=0%7e11135%7e

Please click on the corresponding Trusts tab to view the information.

We trust these details will assist with your enquiries. If we can be of any further assistance in the future please do not hesitate to contact us again.

Questionnaire

As a Trust we are keen to monitor and improve the services we offer. We'd be grateful if you could take the time to answer the following questions and provide us with any other feedback that may assist us with this process:

Were you satisfied with the handling of your request?	**YES/NO**

Was your request handled in a timely manner?	**YES/NO**
Were you provided with sufficient information to assist you with your request?	**YES/NO**

Yours Sincerely

Freedom of Information Office

Digital

Northern Care Alliance

Salford/North Manchester/Oldham/Rochdale/Fairfield

Team Tel: **Please note our offices are closed for the foreseeable future. The best way to contact the team is via email or Microsoft teams**

-----Original Message-----
From: PennineFOI atSalford
Sent: 11 June 2021 07:54
Subject: RE: Freedom of information - TL1S - FOI 11035 - ack

Good morning,

With reference to your request made under the Freedom of Information Act, Salford Royal NHS Foundation Trust acknowledges receipt of your request for information and informs you that the process has been instigated. Our Reference number should be used in all future correspondence.

In accordance with Trust policy and the requirements of the Freedom of Information Act 2000, a period of 20 working days is assigned for processing your request. [Working days within the NHS refers to Mon-Fri.] We will provide you with an explanation if we find that there is any reason why this period may extend beyond the period prescribed by the Act.

Please contact us if you have any queries regarding the procedure.

Kind regards,

Freedom of Information Office

Digital

Northern Care Alliance

Salford/North Manchester/Oldham/Rochdale/Fairfield

Team Tel: Please note our offices are closed for the foreseeable future. The best way to contact the team is via email or Microsoft teams

-----Original Message-----

From: Ryan Kate (Microbiology) <kate.ryan@srft.nhs.uk>

Sent: 10 June 2021 10:31

Cc: Freedom of information request <FreedomOf.InformationRequest@srft.nhs.uk>

Subject: RE: Freedom of information - TL1S - FOI 11035

I have referred your request to the Freedom of Information department.

Kind Regards,

Kate Ryan

Microbiology Service Manager

Pathology at Wigan and Salford (PAWS)

Salford Royal NHS Foundation Trust

Salford Care Organisation

Part of the Northern Care Alliance NHS Group

tel: 0161 206 5025 (Internal extension: 65025)

mobile: 07970268833

Trust email: kate.ryan@srft.nhs.uk

NHS email: paws.microbiology@nhs.net

PA for Microbiology – Diane Lancaster (0161 206 5030 diane.lancaster@srft.nhs.uk)

-----Original Message-----

Sent: 09 June 2021 21:02

To: Ryan Kate (Microbiology) <kate.ryan@srft.nhs.uk>

Subject: Freedom of information

Hi Kate,

Under the freedom of information Act I would like to know the number of cycles you have been using on the PCR test (Polymerase Chain Reaction) test as standard, and if that number has been changed at any time for whatever reason.

I would also like to know how many children under the age of 16 have been logged as a death from SARSCoV2, without any underlying health issues.

And can you tell me if you have any records of SARSCoV2 going through Koch's postulates?

Kind regards

The UK

Sent from my iPhone

3 attachments

PCR Testing - 09 07 2021.pdf
194K

NCA FOI COVID 19 responses 080721.pdf
258K

NCA FOI Response Report - FOI 11135 - Final.pdf
154K

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Freedom of Information Report **FOI / 11135**

Question (dated:09/06/2021)
Requestor Category: Individual

I would like to know the number of cycles you have been using on the PCR (polymerase chain reaction) test as standard and if that number has ever been changed at any time for whatever reason.

I would also like to know how many children with under the age of 16 have been logged as a death from SARSCoV2 without any underlying health issues.

Can you also tell me if you have any records of SARSCoV2 going through Koch's Postulates.

Response – Salford Royal NHS Foundation Trust

All COVID-19 related information for the Trust is published online (including PCR testing). In line with section 21 of the Freedom of Information Act, please visit the link below to access the information requested

https://www.srft.nhs.uk/about-us/freedom-of-information/randr/?entryid34=217656&q=0%7epcr%7e

https://www.srft.nhs.uk/about-us/freedom-of-information/randr/?esctl2071478directoryviewpager_p=1&entryid34=205243&q=0%7efoi+covid%7e

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Salford | Oldham | Bury | Rochdale | North Manchester

Freedom of Information Report **COVID-19 RELATED FIGURES**

COVID- 19 death figures are reported daily on the NHS England website. In line with section 21 of the Freedom of Information Act, please visit the link below to access the information requested

https://www.england.nhs.uk/statistics/statistical-work-areas/covid-19-daily-deaths/

- the number of patients in hospital with COVID including those in mechanical ventilation
- the number of patients admitted to hospital with COVID
- the number of patients diagnosed in hospital with COVID
- the number of patients discharged from hospital and
- staffing absences
- hospital admissions,
- number of Adult G&A beds; occupied by COVID patients; occupied by non-COVID patients, unoccupied
- number of all beds occupied by COVID patients
- number of MV beds occupied by COVID patients

are also reported via the link below

https://www.england.nhs.uk/statistics/statistical-work-areas/covid-19-hospital-activity/

Cause of death is not recorded on our clinical systems and would be recorded on an individual's death certificate. To review all death certificates issued over this time period to establish the primary cause of death is estimated will take in excess of time expectations of the Freedom of Information Act. The Trust therefore sites section 12(1) and is unable to provide a response to this request.

Break down per Care Organisation and Hospital

	Patients (March 2020 – December 2020)	Patients (Jan 2021 – March 2021
Bury and Rochdale Care Organisation (Fairfield General Hospital and Rochdale Infirmary)	12	12
Oldham Care Organisation (The Royal Oldham Hospital)	16	35
North Manchester Care Organisation (North Manchester General Hospital)	88	<10

The total number of patients who have died with a positive COVID-19 test within 28 days of their death with no previous existing health conditions between March 2020 and 8th December 2020 at **Salford Royal NHS Foundation Trust** is **<10**

The total number of patients who have died with a positive COVID-19 test within 28 days of their death with no previous existing health conditions between January 2021 and March 2021 at **Salford Royal NHS Foundation Trust** is **<10**

The Trust applies an exemption under section 41 (1) of the Freedom of Information Act (Information provided in confidence in relation to patients that are deceased and are not afforded rights under the GDPR) and has not provided any figures less than 10.

The Trust is unable to differentiate between those who died with or from COVID-19. However, in the spirit of the FOI Act, the Trust is able to provide the following information of the total number of patients who have died with a positive COVID-19 test within 28 days of their death:

432 patient's deaths have been submitted into the Covid-19 Patient Notification System (CPNS) via NHS England within the time frame of March 2020 to the 16th of December 2020 at **Fairfield General Hospital**

155 patient's deaths have been submitted into the Covid-19 Patient Notification System (CPNS) via NHS England within the time frame of March 2020 to the 22nd of January 2021 at the **Royal Oldham Hospital**

***Figures for April to July 2021 will be published by the end of September 2021 (exempt under section 22 of the FOI Act – intended for future publication)*

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Freedom of Information Report FOI / 11128

Question (dated:09/06/2021)
Requestor Category: Individual

1. I would like to know the number of cycles you have been using on PCR (Polymerase Chain Reaction) test as standard and if that number has ever been changed at anytime for whatever reason.

2. I would also like to know how many children under the age of 16 have been logged as a death from SARSCov2 without any underlying health issues.

3. And can you tell me if you have any records of SARSCov2 going through Koch's Postulates.

Response – Salford Royal Foundation Trust

1. I would like to know the number of cycles you have been using on PCR (Polymerase Chain Reaction) test as standard and if that number has ever been changed at anytime for whatever reason.

   The Trust uses the following Commercial CE IVD assays - Hologic Panther, Cepheid, BD Max, Abbott Alinity, Abbott M2000 and AusDiagnostics Hi-Plex. The number of thermal cycles across all the platforms in use varies slightly and the maximum is 42; the parameters for all these assays are available from the commercial suppliers. The Trust does not alter them for any reason.

2. I would also like to know how many children under the age of 16 have been logged as a death from SARSCov2 without any underlying health issues.

   There were no children under the age of 16 who died within 28 days of a positive COVID-19 test result.

3. And can you tell me if you have any records of SARSCov2 going through Koch's Postulates.

   The Trust has not recorded any cases going through Koch's Postulates.

Response – Pennine Acute Hospitals NHS Trust

1. I would like to know the number of cycles you have been using on PCR (Polymerase Chain Reaction) test as standard and if that number has ever been changed at anytime for whatever reason.

   The Trust uses the following Commercial CE IVD assays - Hologic Panther, Cepheid, BD Max, Abbott Alinity, Abbott M2000 and AusDiagnostics Hi-Plex. The number of thermal cycles across all the platforms in use varies slightly and the maximum is 42; the parameters for all these assays are available from the commercial suppliers. The Trust does not alter them for any reason.

2. I would also like to know how many children under the age of 16 have been logged as a death from SARSCov2 without any underlying health issues.

   The Trust is unable to provide figures of those who have died from COVID-19. In the spirit of the FOI Act, the Trust can provide information on those with a positive COVID 19 test within 28 days of death.
   There were less than 10 children who died within 28 days of a positive COVID-19 test result.
   The Trust applies an exemption under section 41 (1) of the Freedom of Information Act (Information provided in confidence in relation to patients that are deceased and are not afforded rights under the GDPR) and has not provided any figures less than 10.

3. And can you tell me if you have any records of SARSCov2 going through Koch's Postulates.

   The Trust has not recorded any cases going through Koch's Postulates.

September 18, 2021

Public Health England
Wellington House
133-155 Waterloo Road
London, SE1 8UG

www:public-health-england

foi@phe.gov.uk

This is my wish for access to records.

Description of Requested Records:

All studies and/or reports and/or records in the possession, custody or control of the addressed body corporate describing the **purification** (i.e. via filtration and ultra-centrifugation and chromatography) of any **"COVID-19 virus**" (aka "SARS-COV-2", including any alleged "variants" i.e. "B.1.1.7", "B.1.351", "P.1") directly from a sample taken from a diseased man, where the patient sample was not first combined with any other source of **genetic** material (i.e. monkey kidney cells aka Vero cells; fetal bovine serum, liver cancer cells) and also studies and/or reports and/or records in the possession, custody or control of the addressed body corporate proving a causal link between Sars-Cov-2 and the suspected infectious disease Covid-19.

Please note that I am not requesting studies/reports where researchers failed to **purify** the suspected "virus" (separate the alleged "virus" from everything thing else in the patient sample) and instead:

- cultured an unpurified sample or other unpurified substance, and/or
- performed an amplification test (i.e. a PCR test) on the total RNA from a patient sample or from a cell culture, or on genetic material from any unpurified substance, and/or
- fabricated a genome based on PCR-detected sequences in the total RNA from a patient sample or from a cell culture or from any unpurified substance, and/or
- produced electron microscopy images of unpurified things in a cell culture.

Clarification of Request

For further clarity, please note I am already aware that according to virus theory a "virus" requires host cells in order to replicate, and I am **not** requesting records describing the **replication** of a "virus" without host cells.

Further, I am **not** requesting private patient information, or records that describe a suspected "virus" floating in a vacuum; I am simply requesting records that describe its **purification** (**separation** from everything else in the patient sample, as per standard laboratory practices for the purification of other very small things).

Please note that my request includes any study/report matching the above description, for example (but not limited to) any published peer-reviewed study **authored by**

anyone, anywhere.

If any records match the above description of requested records and are currently available in the public domain, please provide enough information about each record so that I may identify and access each one with certainty (i.e. title, author(s), date, journal, where the public may access it). Please provide URLs where possible.

Format:
Pdf documents sent to me via email; I do not wish for anything to be shipped to me.

Contact Information:
Last name: hobbs
First name: yvonne
Address: 33, Lea Close, Broughton Astley, LE9 6NW
Phone:
Email: bastleyellenine@protonmail.com

Public Health England

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Public Accountability Unit
Wellington House
133-155 Waterloo Road
London SE1 8UG

www.gov.uk/phe

By email

[redacted]

Our ref: 18/09/21/ag/1345

20 September 2021

Dear Yvonne Hobbs,

Re: Covid-19

Thank you for your request received on 18 September 2021 addressed to Public Health England (PHE). In accordance with Section 1(1)(a) of the Freedom of Information Act 2000 (the Act), I can confirm that PHE does not hold the information you have specified.

Request

All studies and/or reports and/or records in the possession, custody or control of the addressed body corporate describing the purification (i.e. via filtration and ultra-centrifugation and chromatography) of any "COVID-19 virus" (aka "SARS-COV-2", including any alleged "variants" i.e. "B.1.1.7", "B.1.351", "P.1") directly from a sample taken from a diseased man, where the patient sample was not first combined with any other source of genetic material (i.e. monkey kidney cells aka Vero cells; fetal bovine serum, liver cancer cells) and also studies and/or reports and/or records in the possession, custody or control of the addressed body corporate proving a causal link between Sars-Cov-2 and the suspected infectious disease Covid-19.
Please note that I am not requesting studies/reports where researchers failed to purify the suspected "virus" (separate the alleged "virus" from everything thing else in the patient sample) and instead:

- ***cultured an unpurified sample or other unpurified substance, and/or***
- ***performed an amplification test (i.e. a PCR test) on the total RNA from a patient sample or from a cell culture, or on genetic material from any unpurified substance, and/or***
- ***fabricated a genome based on PCR-detected sequences in the total RNA from a patient sample or from a cell culture or from any unpurified substance, and/or***

produced electron microscopy images of unpurified things in a cell culture

In accordance with Section 1(1)(a) of the FOI Act, PHE can confirm that it does not holds the information you have requested.

Viruses are not independent living entities. They require a host cell substrate to replicate.

PHE's microbiology teams use the term "isolation" to mean culture in the laboratory. It is used sometimes interchangeably to mean isolation from a patient or clinical material – but usually implies that the organism has been grown in culture. An organism is identified by looking for its unique genetic material in a clinical sample and further identification is refined and confirmed by whole genome sequencing.

The Virus Reference Laboratory at PHE, Colindale, London has grown the virus, SARS-CoV-2. The virus culture method has been published in the following peer-reviewed paper: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.32.2001483

Several strains of the SARS-CoV-2 virus has been deposited to the European Virus Archive by PHE.

PHE's culture work on other SARS-CoV-2 variants is in progress and has not been published in any peer-reviewed papers at present.

Under Section 16, a public authority has a duty to provide advice and assistance. Accordingly, please find the following links below regarding evidence of COVID-19:

- SARS-CoV-2 has been cultured and then subjected to electron microscopy. Evidence of the Electron Micrograph is available at the following link: https://publichealthmatters.blog.gov.uk/2021/02/05/what-do-we-know-about-the-new-covid-19-variants/

- General information pertaining to SARS-CoV-2, which causes the disease known as COVID-19: https://www.gov.uk/government/publications/wuhan-novel-coronavirus-background-information/wuhan-novel-coronavirus-epidemiology-virology-and-clinical-features

If you have any queries regarding the information that has been supplied to you, please refer your query to me in writing in the first instance. If you remain dissatisfied and would like to request an internal review, then please contact us at the address above or by emailing foi@phe.gov.uk.

Please note that you have the right to an independent review by the Information Commissioner's Office if a complaint cannot be resolved through the PHE complaints procedure. The Information Commissioner's Office can be contacted by calling the ICO's helpline on 0303 123 1113, visiting the ICO's website at www.ico.org.uk or writing to the ICO at Wycliffe House, Water Lane, Wilmslow, Cheshire, SK9 5AF.

Yours sincerely
FOI Team

Public Health England

Public Accountability Unit
Wellington House
133-155 Waterloo Road
London SE1 8UG
www.gov.uk/phe

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By email
lewie.mm@outlook.com

Our ref: 11/08/21/ag/1028

07 September 2021

Dear Lewis Murphy-Munroe,

Re: FOI request

Thank you for your request received on 11 August 2021 addressed to Public Health England (PHE). In accordance with Section 1(1)(a) of the Freedom of Information Act 2000 (the Act), I can confirm that PHE does not hold the information you have specified.

Request

I am filing a challenge to my public health act by writing to request information of 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 (ie. money kidney cells aka vero cells; liver cancer cells).

I am using "isolation" in the everyday 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.

To conclude, I am requesting all such records that are in the possession, custody or control of Public Health England (for example: downloaded to a computer, printed in hard copy, etc.).

Response

In accordance with Section 1(1)(a) of the FOI Act, PHE can confirm that it does not hold the information you have requested.

Viruses are not independent living entities. They require a host cell substrate to replicate.

PHE's microbiology teams use the term "isolation" to mean culture in the laboratory. It is used sometimes interchangeably to mean isolation from a patient or clinical material – but usually implies that the organism has been grown in culture. An organism is identified by looking for its unique genetic material in a clinical sample and further identification is refined and confirmed by whole genome sequencing.

The Virus Reference Laboratory at PHE, Colindale, London has grown the virus, SARS-CoV-2. The virus culture method has been published in the following peer-reviewed paper: https://www.eurosurveillance.org/content/10.2807/1560-

Response

...dance with Section 1(1)(a) of the FOI Act, PHE can confirm that it does not ... information you have requested.

...are not independent living entities. They require a host cell substrate to replicate.

PHE's microbiology teams use the term "isolation" to mean culture in the laboratory. It is used sometimes interchangeably to mean isolation from a patient or clinical material – but usually implies that the organism has been grown in culture. An organism is identified by looking for its unique genetic material in a clinical sample and further identification is refined and confirmed by whole genome sequencing.

The Virus Reference Laboratory at PHE, Colindale, London has grown the virus, SARS-CoV-2. The virus culture method has been published in the following peer-reviewed paper: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.32.2001483

Several strains of the SARS-CoV-2 virus has been deposited to the European Virus Archive by PHE.

PHE's culture work on other SARS-CoV-2 variants is in progress and has not been published in any peer-reviewed papers at present.

Under Section 16, a public authority has a duty to provide advice and assistance. Accordingly, please find the following links below regarding evidence of COVID-19:

- SARS-CoV-2 has been cultured and then subjected to electron microscopy. Evidence of the Electron Micrograph is available at the following link: https://publichealthmatters.blog.gov.uk/2021/02/05/what-do-we-know-about-the-new-covid-19-variants/

- General information pertaining to SARS-CoV-2, which causes the disease known as COVID-19: https://www.gov.uk/government/publications/wuhan-novel-coronavirus-background-information/wuhan-novel-coronavirus-epidemiology-virology-and-clinical-features

If you have any queries regarding the information that has been supplied to you, please refer your query to me in writing in the first instance. If you remain dissatisfied and would like to request an internal review, then please contact us at the address above or by emailing foi@phe.gov.uk.

Please note that you have the right to an independent review by the Information Commissioner's Office if a complaint cannot be resolved through the PHE complaints procedure. The Information Commissioner's Office can be contacted by calling the ICO's helpline on 0303 123 1113, visiting the ICO's website at www.ico.org.uk or writing to the ICO at Wycliffe House, Water Lane, Wilmslow, Cheshire, SK9 5AF.

Yours sincerely
FOI Team

Public Health England

Protecting and improving the nation's health

Public Accountability Unit
Wellington House
133-155 Waterloo Road
London SE1 8UG

T 020 8327 6920

www.gov.uk/phe

By email
request-701311-306ec32d@whatdotheyknow.com

Our ref: 26/10/ld/1740

3 November 2020

Dear Athanasios Kandias,

Re: 1740 – FOI NIBSC records on SARS-COV-2

Thank you for your email dated 26 October 2020. In accordance with Section 1(1)(a) of the Freedom of Information Act 2000 (the Act), I can confirm that Public Health England does not hold the information you have specified.

Request

All records in the possession, custody or control of NIBSC, 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; liver cancer cells).

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 the NIBSC or that pertain to work done by the NIBSC. My request includes any sort of record, for example (but not limited to) any published peer-reviewed study that the NIBSC has downloaded or printed.

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).

Response

PHE can confirm it does not hold the information you have specified.

Under Section 16 of the Act, public authorities have a duty to provide advice and assistance. I have signposted you to the below links which contain information on taking COVID-19 swabs:

https://www.gov.uk/government/publications/covid-19-guidance-for-taking-swab-samples
https://www.gov.uk/government/publications/types-and-uses-of-coronavirus-covid-19-tests/types-and-uses-of-coronavirus-covid-19-tests

Additionally, the below publication contains some information on virus isolation:
https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.32.2001483

If you have any queries regarding the information that has been supplied to you, please refer your query to in writing in the first instance. If you remain dissatisfied and would like to request an internal review, then please contact us at the address above or by emailing foi@phe.gov.uk.

Please note that you have the right to an independent review by the Information Commissioner's Office if a complaint cannot be resolved through the PHE complaints procedure. The Information Commissioner's Office can be contacted by writing to Information Commissioner's Office, Wycliffe House, Water Lane, Wilmslow, Cheshire, SK9 5AF.

Yours sincerely,
FOI Team

Public Health England

Protecting and improving the nation's health

Public Accountability Unit
Wellington House
133-155 Waterloo Road
London SE1 8UG

T 020 8327 6920

www.gov.uk/phe

By email
request-679711-9694b4f1@whatdotheyknow.com

Our ref: 25/07/hf/878

20 August 2020

Dear Marc Horn,

Re: Full, accurate and complete disclosure of SARS-COV-2 virus isolation records

Thank you for your email dated 25 July 2020. In accordance with Section 1(1)(a) of the Freedom of Information Act 2000 (the Act), I can confirm that Public Health England (PHE) does/does not the information you have specified. I have set out PHE's response to your questions below.

Your Request

Please provide a full, accurate and complete list of records held within your office, and or under your authority, describing the isolation of a SARS-COV-2 virus, directly taken from a symptomatic patient of COVID-19 where the sample was not first combined with any other source of genetic material (not limited but by way of example monkey kidney cells, aka vero cells, liver cancer cells) thereby eliminating contamination as a possible alternative source of sampling.
Please note isolation is used in the normally understood meaning of the word – the act of separating a thing from another. I am not referring, and hence not requesting, to isolation meaning the culture of something else, the performance of an amplification test (eg PCR test which only detect mRNA or DNA) or the sequencing of "something".
If any records match the above description and are available to the public elsewhere, please provide enough information so that I may identify and access each record with certainty (i.e. title, author(s), date, journal, and weblink or location where the public may access it).

Response

PHE can confirm is does not hold information in the way suggested by your request.

Under section 16 of the Act, public authorities have a duty to provide advice and assistance. I have signposted you to the below links which contain information on taking COVID-19 swabs.
https://www.gov.uk/government/publications/covid-19-guidance-for-taking-swab-samples

https://www.gov.uk/government/publications/types-and-uses-of-coronavirus-covid-19-tests/types-and-uses-of-coronavirus-covid-19-tests

Additionally, the below publication contains some information on virus isolation: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.32.2001483

If you have any queries regarding the information that has been supplied to you, please refer your query to in writing in the first instance. If you remain dissatisfied and would like to request an internal review, then please contact us at the address above or by emailing foi@phe.gov.uk.

Please note that you have the right to an independent review by the Information Commissioner's Office if a complaint cannot be resolved through the PHE complaints procedure. The Information Commissioner's Office can be contacted by writing to Information Commissioner's Office, Wycliffe House, Water Lane, Wilmslow, Cheshire, SK9 5AF.

Yours sincerely,
FOI Team

Public Health England

Protecting and improving the nation's health

Public Accountability Unit
Wellington House
133-155 Waterloo Road
London SE1 8UG

T 020 8327 6920

www.gov.uk/phe

By email
request-680379-1a327a9f@whatdotheyknow.com

Our ref: 28/07/cs/904

25 August 2020

Dear Marc Horn,

Re: Documents held showing SARS-COV2 has been isolated and Causes COVID-19

Thank you for your email dated 28 July 2020. In accordance with Section 1(1)(a) of the Freedom of Information Act 2000 (the Act), I can confirm that Public Health England (PHE) does not hold the information you have specified.

Your Request

Please provide a full, accurate and complete list of records held within your office, and / or under your authority, supporting the claim that the SARS-COV-2 virus causes the symptoms of the disease called COVID-19, including but not limited to isolation of SARS-COV-2 virus and its identification method as confirmation.

Please note isolation is used in the normally understood meaning of the word – the act of separating a thing from another. I am not referring to, and hence not requesting, isolation meaning the culture of something else or the performance of an amplification test (eg PCR test which only detect mRNA or DNA) or the sequencing of "something".

If any records match the above description and are available to the public elsewhere, please provide enough information so that I may identify and access each record with certainty (i.e. title, author(s), date, journal, and weblink or location where the public may access it).

Response

Evidence that SARS-COV-2 causes the symptoms of COVID-19 is widely and publicly available.

Under section 16 of the Act, public authorities have a duty to provide advice and assistance. I have signposted you to publications that contain information on virus isolation:

www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.32.2001483

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

www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30154-9/fulltext.

If you have any queries regarding the information that has been supplied to you, please refer your query to in writing in the first instance. If you remain dissatisfied and would like to request an internal review, then please contact us at the address above or by emailing foi@phe.gov.uk.

Please note that you have the right to an independent review by the Information Commissioner's Office if a complaint cannot be resolved through the PHE complaints procedure. The Information Commissioner's Office can be contacted by writing to Information Commissioner's Office, Wycliffe House, Water Lane, Wilmslow, Cheshire, SK9 5AF.

Yours sincerely,
FOI Team

Public Health England

Protecting and improving the nation's health

Public Accountability Unit
Wellington House
133-155 Waterloo Road
London SE1 8UG

www.gov.uk/phe

By email

[redacted]

Our ref: 11/09/21/ag/1287

28 September 2021

[redacted]

Re: Purification of SARS-COV-2 and Variants

Thank you for your request received on 11 September 2021 addressed to Public Health England (PHE). In accordance with Section 1(1)(a) of the Freedom of Information Act 2000 (the Act), I can confirm that PHE does not hold the information you have specified.

Request

All studies and/or reports in the possession, custody or control of Public Health England describing the purification of any "COVID-19 virus" (aka "SARS-COV-2", including any alleged "variants" i.e. "B.1.1.7", "B.1.351", "P.1") (for example: via filtration, ultracentrifugation and chromatography), directly from a sample taken from a diseased human where the patient sample was not first combined with any other source of genetic material (i.e. monkey kidney cells aka Vero cells; fetal bovine serum).

Please note that I am not requesting studies/reports where researchers failed to purify the suspected "virus" and instead:

- ***cultured an unpurified sample or other unpurified substance, and/or***
- ***performed an amplification test (i.e. a PCR test) on all the RNA from a patient sample or from a cell culture, or on genetic material from any unpurified substance, and/or***
- ***fabricated a "genome" by editing/assembling/aligning sequences detected in the total RNA from a patient sample or from a cell culture or from any unpurified substance, and/or***
- ***produced electron microscopy images of unpurified things.***

In accordance with Section 1(1)(a) of the FOI Act, PHE can confirm that it does not hold the information you have requested.

Viruses are not independent living entities. They require a host cell substrate to replicate.

PHE's microbiology teams use the term "isolation" to mean culture in the laboratory. It is used sometimes interchangeably to mean isolation from a patient or clinical

material – but usually implies that the organism has been grown in culture. An organism is identified by looking for its unique genetic material in a clinical sample and further identification is refined and confirmed by whole genome sequencing.

The Virus Reference Laboratory at PHE, Colindale, London has grown the virus, SARS-CoV-2. The virus culture method has been published in the following peer-reviewed paper: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.32.2001483

Several strains of the SARS-CoV-2 virus has been deposited to the European Virus Archive by PHE.

PHE's culture work on other SARS-CoV-2 variants is in progress and has not been published in any peer-reviewed papers at present.

Under Section 16, a public authority has a duty to provide advice and assistance. Accordingly, please find the following links below regarding evidence of COVID-19:

- SARS-CoV-2 has been cultured and then subjected to electron microscopy. Evidence of the Electron Micrograph is available at the following link: https://publichealthmatters.blog.gov.uk/2021/02/05/what-do-we-know-about-the-new-covid-19-variants/

- General information pertaining to SARS-CoV-2, which causes the disease known as COVID-19: https://www.gov.uk/government/publications/wuhan-novel-coronavirus-background-information/wuhan-novel-coronavirus-epidemiology-virology-and-clinical-features

If you have any queries regarding the information that has been supplied to you, please refer your query to me in writing in the first instance. If you remain dissatisfied and would like to request an internal review, then please contact us at the address above or by emailing foi@phe.gov.uk.

Please note that you have the right to an independent review by the Information Commissioner's Office if a complaint cannot be resolved through the PHE complaints procedure. The Information Commissioner's Office can be contacted by calling the ICO's helpline on 0303 123 1113, visiting the ICO's website at www.ico.org.uk or writing to the ICO at Wycliffe House, Water Lane, Wilmslow, Cheshire, SK9 5AF.

Yours sincerely
FOI Team

Public Health England

Protecting and improving the nation's health

Public Accountability Unit
Wellington House
133-155 Waterloo Road
London SE1 8UG

T 020 8327 6920

www.gov.uk/phe

By email

[redacted]

Our ref: 04/09/kl/1184

21 September 2020

Dear [redacted]

Re: Freedom of Information Request: Studies re Isolation of SARS-COV-2

Thank you for your email dated 4 September 2020. In accordance with Section 1(1)(a) of the Freedom of Information Act 2000 (the Act), I can confirm that Public Health England (PHE) does not hold the information you have specified.

Your Request

All records in the possession, custody or control of Public Health England 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 Public Health England or that pertain to work done by Public Health England. My request includes any sort of record, for example (but not limited to) any published peer-reviewed study that Public Health England 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).

Response

PHE can confirm it does not hold information in the way suggested by your request.

Under Section 16 of the Act, public authorities have a duty to provide advice and assistance. I have signposted you to the below links which contain information on taking COVID-19 swabs:
https://www.gov.uk/government/publications/covid-19-guidance-for-taking-swab-samples
https://www.gov.uk/government/publications/types-and-uses-of-coronavirus-covid-19-tests/types-and-uses-of-coronavirus-covid-19-tests

Additionally, the below publication contains some information on virus isolation:
https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.32.2001483

If you have any queries regarding the information that has been supplied to you, please refer your query to in writing in the first instance. If you remain dissatisfied and would like to request an internal review, then please contact us at the address above or by emailing foi@phe.gov.uk.

Please note that you have the right to an independent review by the Information Commissioner's Office if a complaint cannot be resolved through the PHE complaints procedure. The Information Commissioner's Office can be contacted by writing to Information Commissioner's Office, Wycliffe House, Water Lane, Wilmslow, Cheshire, SK9 5AF.

Yours sincerely,
FOI Team

[redacted] 2-9-2020 08:55

document request SARS-CoV-2

Aan access.to.documents@ecdc.europa.eu <access.to.documents@ecdc.europa.eu>

Dear sir/madam,

Please provide me with the following:

1) A single document that proves, scientifically, that SARS-CoV-2 exists and that proves that the genetic sequence of SARS-CoV-2, used in the RT-PCR tests is specific for SARS-CoV-2 only.

2) A document (name, number, date) that describes the scientific procedure, or methodology that is required to be followed by the ECDC as part of the quality standard to prove that a virus exists.

3) A document that provides an assessment by the ECDC that shows that 1) complies with 2) for SARS-CoV-2

kind regards,

[redacted]

[redacted]

Stockholm, 16 September 2020
Our ref.: DPR-2020-OUT-3176-KEElKh

Dear Mr [redacted],

Re: Your application for access to documents – Ref 20-3696

We refer to your email dated 31 August 2020 in which you make a request for access to documents, registered on 1 September 2020 under the above mentioned reference number, and your follow up email on 2 September 2020 that has been handled under the same reference number as well.

We regret to inform you that no documents were found that would correspond to the description given in your application.

Indeed, as specified in Article 2(3) of Regulation 1049/2001, the right of access as defined in that Regulation applies only to existing documents in the possession of the institution.

Given that no such documents have been identified, ECDC is not in a position to handle your request.

However, in the spirit of The European Code of Good Administrative Behaviour, we take the liberty of suggesting the following links to some information on this topic that you might find useful:

- Regarding the "aetiology of SARS: Koch's postulates fulfilled":
https://covid19.elsevierpure.com/de/publications/the-aetiology-of-sars-kochs-postulates-fulfilled

- About how to detect and show the sequence phylogeny:
https://www.ecdc.europa.eu/en/novel-coronavirus/laboratory-support

Additionally, among others, we would like to refer to two relevant seminal papers; on the virus discovery and on the first RT-PCR development, which also includes an investigation of specificity, which excludes unspecific detection of e.g. seasonal coronaviruses. Please see the links below:

https://www.nature.com/articles/s41586-020-2012-7

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6988269/

European Centre for Disease Prevention and Control (ECDC)
Gustav den III:s Boulevard 40, 169 73 Solna, Sweden
Phone: +46 (0)8 58 60 10 00 - Fax: +46 (0)8 58 60 10 01
www.ecdc.europa.eu

In accordance with Article 7(2) of Regulation 1049/2001, you are entitled to make a confirmatory application requesting ECDC's Director to review this position.

Such a confirmatory application should be addressed within 15 working days upon receipt of this letter to the following address:

ECDC
Legal Services
Gustav III:s Boulevard 40
16973 Solna
Sweden

or by email to: confirmatory.requests@ecdc.europa.eu.

Yours faithfully,

Karl Ekdahl
Head of Unit Disease Programmes

European Centre for Disease Prevention and Control (ECDC)
Gustav den III:s Boulevard 40, 169 73 Solna, Sweden
Phone: +46 (0)8 58 60 10 00 - Fax: +46 (0)8 58 60 10 01
www.ecdc.europa.eu

[redacted] 1-10-2020 09:25

confirmatory application Ref 20-3696

Aan confirmatory.requests@ecdc.europa.eu <confirmatory.requests@ecdc.europa.eu>

Dear sir/madam,

In your response to my information request, which has been registered under reference number Ref 20-3696, i would like to ask for a confirmatory application and request the ECDC's Director to review the position stated in you letter with reference DPR-2020-OUT-3176-KEElKh.

First, you have not answered any of my questions and have send me references to research papers that do not answer any of my questions. I would like therefore to ask you for a truthful answer to the following.

In question 1) I have asked for a single document that proves, scientifically, that SARS-CoV-2 exists and that proves that the genetic sequence of SARS-CoV-2, used in the RT-PCR tests is specific for SARS-CoV-2 only.

A) Can you please confirm that at present, the ECDC does not have any scientific proof of the existence of SARS-CoV-2?

B) If you do have the opinion that SARS-CoV-2 exists, please provide me with a reference from which the ECDC considers that this has been proven. Please also provide a thorough assessment, based on the quality standards of the ECDC, why this scientific paper fulfills the requirements of having discovered a new virus.

In question 2) I ask for a document (name, number, date) that describes the scientific procedure, or methodology that is required to be followed by the ECDC as part of the quality standard to prove that a virus exists.

C) Please confirm that the ECDC does not have a quality standard in which the methodology of proving the existence of a virus and the procedures to quantify the biochemical properties of the new virus has been defined. If you do have a quality standard, please send it to me.

3) A document that provides an assessment by the ECDC that shows that 1) complies with 2) for SARS-CoV-2

D) If not available, please confirm that the ECDC has not executed an assessment to verify whether or not SASRS-CoV-2 exists.

Kind regards,

[redacted]

[redacted]

Stockholm, 21 October 2020
Our ref.: DIR-2020-OUT-3783-AAElKh

Dear Mr [redacted],

Re: Your confirmatory application for access to documents – Ref 20-3696-1

We refer to your email dated 1 October 2020 registered on the same day under the above mentioned reference number. In your email you make a confirmatory application with regards to our letter DPR-2020-OUT-3176-KEElEKh of 16 September 2020, replying to your initial application of 31 August and 2 September 2020.

I can confirm that ECDC does not hold any document corresponding to the description you made in your initial application.

In your confirmatory application, you also ask ECDC to provide additional information to you, or to confirm certain assumptions that you make (points A, B, C and D of your email). Such request falls outside the scope of the confirmatory application and in general of Regulation 1049/2001, and I will deal with them instead as a request for information, processed in accordance with the ECDC Code on Good Administrative Behaviour.

On this respect, I bring your attention to the fact that, in accordance with paragraph 76 of the Judgment of the Court of first Instance of 25 April 2007 in case T-264/04, WWF European Policy Programme v Council,

The public's right of access to the documents of the institutions covers only documents and not information in the wider meaning of the word and does not imply a duty on the part of the institutions to reply to any request for information from an individual

The same paragraph explicitly states that access to information *may be granted only if that information is contained within documents, which presupposes that such documents exist.*

While ECDC strives to be close to the European citizens, in line with the Code of Good Administrative Behaviour, the principle of sound administration obliges me, in particular in this time of pandemic, to focus all the resources of the Agency to tasks that I believe can have a significant impact for public health, in accordance with the ECDC mission.
In view of all the above, and taking into account that ECDC already provided you with relevant information in our letter of 16 September, I decided that ECDC will not reply to the further questions that you included in your email of 1 October 2020, and that ECDC shall discontinue any further correspondence with you

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related to the issues that you mention, as I consider that any further correspondence would be repetitive and pointless.

Remedies

You can bring an action to the Court of Justice of the European Union against the part of this decision concerning the confirmatory application, in accordance with art. 263 of the Treaty on the Functioning of the European Union. You also can lodge a complaint to the European Ombudsman, in accordance with art. 228 of the Treaty on the Functioning of the European Union.

Yours faithfully,

Andrea Ammon
Director

European Centre for Disease Prevention and Control (ECDC)
Gustav den III:s Boulevard 40, 169 73 Solna, Sweden
Phone: +46 (0)8 58 60 10 00 - Fax: +46 (0)8 58 60 10 01
www.ecdc.europa.eu

FOI Request to Finland's Institute for Health and Welfare (THL)

and

subsequent complaint to

The Finnish National Board on Research Integrity

Hyvä vastaanottaja,

Tämä on julkisuuslakiin (http://www.finlex.fi/fi/laki/ajantasa/1999/19990621) perustuva tietopyyntö.

Vuonna 2020 WHO julisti maailmanlaajuisen koronaviruspandemian.
Pyydän nähtäväksi ja julkisesti julkaistavaksi todisteet koronaviruksen (Sars-Cov-2) täydellisestä eristämisestä, niin että virus on todella eristetty kaikesta muusta, soluista, kudoksista yms.
Lisäksi pyydän valokuvan eristetystä kyseisestä viruksesta, karakterisaatio sen biokemiallisesta rakenteesta, sen genomi sekvensoituna sekä määrittely mistä proteiineista kyseinen virus koostuu.
Samalla pyydän todisteet siitä, että juuri tuo kyseinen virus aiheuttaa ihmisissä oireita.

Pyydän toimittamaan aineiston jäljennöksen viivytyksettä sähköisessä muodossa liitetiedostona vastauksena tähän viestiin. Tietoaineistot avoimena rakenteellisena datana, eli .xls-, .csv-, .sql-, tai muussa rakenteellisessa muodossa. Dokumentit pyydän uudelleenkäytettävässä muodossa, kuten .doc, odf-, .ppt tai pdf/a-muodossa.
Olisi toivottavaa että aineisto olisi julkisuuslain 20 § mukaan vastedes saatavilla organisaationne web-sivulla.
Pyydän toimittamaan tiedot julkl 16 § mukaisesti pyydetyllä tavalla pyydetyssä muodossa tai perustelemaan sähköpostitse viivytyksettä mikäli on syy toimittaa toisella tavalla.
Huomioittehan että julkl. 34§ mukaan asiakirjan antamisesta ei peritä maksua, kun julkinen sähköisesti talletettu asiakirja lähetetään tiedon pyytäjälle sähköpostitse.
Pyydän toimittamaan tiedot viivytyksettä julkl. 14.4§ mukaisesti, enintään 2 viikon määräajan kuluessa, tai perustelemaan ensi tilassa mikäli tietojen toimittamiseen tarvitaan pidempi kuukauden toimitusaika.
Mikäli pyyntöä ei voida täyttää, pyydän 14.4§ mukaisessa 2 viikon määräajan kuluessa valituskelpoisen päätöksen.
Pyydän viivytyksettä kuittaamaan viestin vastaanotetuksi ja kertomaan asian diaarinumeron.

Ystävällisin terveisin,
[redacted]

Hyvä [redacted]

viitaten 6.9.2021 Terveyden ja hyvinvoinnin laitokselle (THL) lähettämäänne tietopyyntöön (THL/4635/3.10.00/2021), THL ilmoittaa vastauksenaan seuraavaa:

Viruksen eristämisestä puhutaan silloin, kun potilasnäyte istutetaan soluviljelmään ja virus alkaa siinä lisääntyä. Koronavirusdiagnostiikassa viruseristys ei ole rutiinitoimenpide. Viruseristys vaatii erityisturvatason laboratorion ja se on aikaa vievä toimenpide. Suomessa koronaviruksia on eristetty kuitenkin sekä diagnostiikan kehittämistarkoituksiin että virusten ominaisuuksien tutkimista varten. Ohessa tieteellinen julkaisu Suomen ensimmäisestä koronatapauksesta tammikuulta 2020, jolloin koronavirus eristettiin Suomessa ensimmäisen kerran. Sivulla 2 kappaleessa SARS-CoV2/Finland/1/2020 virus isolation kerrotaan erityisesti viruksen eristämisestä soluviljelmässä.

Elektronimikroskooppikuvia koronaviruksesta on runsaasti löydettävissä erilaisista kuvapankeista, esim. https://www.niaid.nih.gov/news-events/novel-coronavirus-sarscov2-images.

Tämän linkin kautta pääsette tarkastelemaan SARS-CoV-2 -viruksen rakennetta: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164637/pdf/367_1260.pdf

Teillä on mahdollisuus saattaa asia viranomaisen ratkaistavaksi ilmoittamalla siitä sähköpostitse THL:n kirjaamoon kirjaamo@thl.fi, jolloin saatte asiasta valituskelpoisen hallintopäätöksen.

Ystävällisin terveisin

Hanna Kaarre

toimeksi saaneena

AUTO-TRANSLATION

Dear Recipient,

This is a request for information based on the Publicity Act (http://www.finlex.fi/fi/laki/ajantasa/1999/19990621).

In 2020, the WHO declared a global coronary virus pandemic. I request that evidence of complete isolation of the coronavirus (Sars-Cov-2) be seen and made public so that the virus is indeed isolated from all other cells, tissues, etc. In addition, a photo of the virus isolated that virus consists of. At the same time, I am asking for evidence that it is that virus that is causing the symptoms in humans.

Please provide a copy of the material in electronic form without delay as an attachment in response to this message. Data sets as open structured data, ie .xls, .csv, .sql, or other structured format. I request documents in a reusable format, such as .doc, odf, .ppt, or pdf / a.

It would be desirable for the material to be available on your organization’s website from now on, in accordance with section 20 of the Public Access to Information Act.

If the request cannot be complied with, I will request an appealable decision within the 2-week period pursuant to section 14.4. Please acknowledge receipt of the message without delay and state the diary number.

Best regards,
[redacted]

Dear [redacted],

referring to your request for information sent to the National Institute for Health and Welfare (THL) on 6.9.2021 (THL / 4635 / 3.10.00 / 2021), THL replies as follows:

Virus isolation is when a patient sample is planted in a cell culture and the virus begins to multiply in it. In coronavirus diagnostics, virus isolation is not a routine procedure. Virus isolation requires a special level of security in the laboratory and is a time consuming operation. In Finland, however, coronaviruses have been isolated both for diagnostic development purposes and for studying the properties of the viruses. Attached is a scientific publication on the first corona case in Finland in January 2020, when the coronavirus was isolated for the first time in Finland. On page 2, section SARS-CoV2 / Finland / 1/2020 virus isolation describes in particular the isolation of the virus in cell culture.

Electron micrographs of the coronavirus can be found in abundance in various image banks, e.g., https://www.niaid.nih.gov/news-events/novel-coronavirus-sarscov2-images.

Use this link to view the structure of the SARS-CoV-2 virus:

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7164637/pdf/367_1260.pdf

You have the opportunity to refer the matter to THL by e-mail to the registry office at kirjaamo@thl.fi, in which case you will receive an appealable administrative decision.

Yours sincerely,

Hanna Kaarre

Rapid communication

Serological and molecular findings during SARS-CoV-2 infection: the first case study in Finland, January to February 2020

Anu Haveri[1] , Teemu Smura[2] , Suvi Kuivanen[2] , Pamela Österlund[1] , Jussi Hepojoki[2,3] , Niina Ikonen[1] , Marjaana Pitkäpaasi[1] , Soile Blomqvist[1] , Esa Rönkkö[1] , Anu Kantele[4] , Tomas Strandin[2] , Hannimari Kallio-Kokko[5] , Laura Mannonen[5] , Maija Lappalainen[5] , Markku Broas[6] , Miao Jiang[1,7] , Lotta Siira[1] , Mika Salminen[1] , Taneli Puumalainen[1] , Jussi Sane[1] , Merit Melin[1] , Olli Vapalahti[2,5] , Carita Savolainen-Kopra[1]

1. Department of Health Security, Finnish Institute for Health and Welfare (THL), Helsinki, Finland
2. University of Helsinki, Medicum, Department of Virology, Helsinki, Finland
3. Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland
4. Inflammation Center, Infectious Diseases, Helsinki University Hospital (HUSLAB) and University of Helsinki, Helsinki, Finland
5. Department of Virology and Immunology, Helsinki University Hospital (HUSLAB) and University of Helsinki, Helsinki, Finland
6. Infection-Hospital Hygiene Unit, Lapland Central Hospital, Rovaniemi, Finland
7. Faculty of Biological and Environmental Sciences, University of Helsinki, Helsinki, Finland

Correspondence: Anu Haveri (anu.haveri@thl.fi)

Citation style for this article:
Haveri Anu , Smura Teemu , Kuivanen Suvi , Österlund Pamela , Hepojoki Jussi , Ikonen Niina , Pitkäpaasi Marjaana , Blomqvist Soile , Rönkkö Esa , Kantele Anu , Strandin Tomas , Kallio-Kokko Hannimari , Mannonen Laura , Lappalainen Maija , Broas Markku , Jiang Miao , Siira Lotta , Salminen Mika , Puumalainen Taneli , Sane Jussi , Melin Merit , Vapalahti Olli , Savolainen-Kopra Carita . Serological and molecular findings during SARS-CoV-2 infection: the first case study in Finland, January to February 2020. Euro Surveill. 2020;25(11):pii=2000266. https://doi.org/10.2807/1560-7917.ES.2020.25.11.2000266

Article submitted on 06 Mar 2020 / accepted on 18 Mar 2020 / published on 19 Mar 2020

The first case of coronavirus disease (COVID-19) in Finland was confirmed on 29 January 2020. No secondary cases were detected. We describe the clinical picture and laboratory findings 3–23 days since the first symptoms. The SARS-CoV-2/Finland/1/2020 virus strain was isolated, the genome showing a single nucleotide substitution to the reference strain from Wuhan. Neutralising antibody response appeared within 9 days along with specific IgM and IgG response, targeting particularly nucleocapsid and spike proteins.

On 31 December 2019, a cluster of pneumonia cases of unknown aetiology was reported in Wuhan, Hubei Province, China [1]. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was isolated by Chinese scientists on 7 January 2020. To date, the SARS-CoV-2 virus causing the coronavirus disease (COVID-19) pandemic is spreading throughout the world.

Here we describe the timeline of events around the first COVID-19 case imported to Finland, and summarise the clinical, molecular and serological data. Successful SARS-CoV-2/Finland/1/2020 isolation enabled us to use the cytopathic effect (CPE)-based microneutralisation (MN) assay to detect SARS-CoV-2-specific neutralising antibody levels. Diagnostic serum samples of the case and three close contacts were analysed and compared with serum samples from the Finnish population collected in 2019.

Clinical presentation and laboratory confirmation of the case

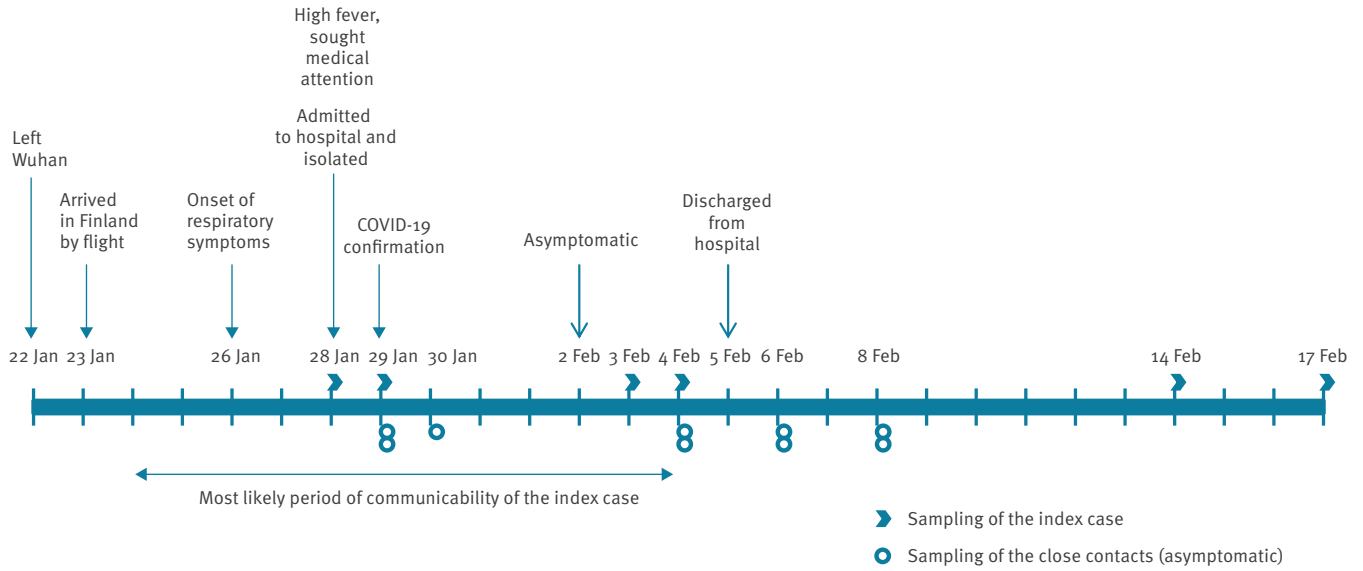
The first COVID-19 case in Finland was a female Chinese tourist in her 30s, who had left Wuhan on 22 January and arrived in Finland on 23 January. Her first symptoms were a runny nose on 26 January and nausea on 27 January. Because of high fever (39 °C), weakness and cough she sought medical attention on 28 January. Suspicion of COVID-19 led to her direct transfer to the Lapland Central Hospital in Rovaniemi, where she was isolated and sampled on 28 and 29 January for laboratory confirmation of SARS-CoV-2 infection (Figure 1). SARS-CoV-2 infection was confirmed from nasopharyngeal samples on 29 January by the Helsinki University Hospital Laboratory (HUSLAB), and further confirmed at the Finnish Institute for Health and Welfare (THL) (Table). Both laboratories performed real-time RT-PCR testing for three targets: the envelope (E), the RNA-dependent RNA polymerase (RdRp) and the nucleocapsid (N). Primers and probes were based on the Corman et al. method [2]. Cycle threshold (Ct) values above 37 were considered negative.

The case had mild symptoms throughout the isolation period. She was tested PCR-negative in 3 and 4 February samples and, as considered asymptomatic, discharged from hospital on 5 February. One additional sample for serology and PCR was taken on 14 and 17 February, respectively.

Altogether 21 close contacts were identified of whom we could reach 17. Fourteen were still in Finland and

Figure 1

Timeline of events around the first COVID-19 case imported to Finland, January–February 2020



COVID-19: coronavirus disease.

placed in quarantine for 14 days. Information about three close contacts that had left the country was communicated to the competent authorities in their respective countries. For the remaining four close contacts, we had no contact details. Two of the 21 close contacts were closely co-exposed and therefore sampled on Days 4, 10, 12 and 14 after the first symptoms of the index case. Follow-up of all contacts ended on 11 February without secondary transmission events.

SARS-CoV-2/Finland/1/2020 virus isolation

The SARS-CoV-2 virus SARS-CoV-2/Finland/1/2020 was isolated in a biosafety level 3 (BSL-3) laboratory in Vero E6 cells from the Day 4 nasopharyngeal swab (NPS) and nasopharyngeal aspirate (NPA) specimens (Table). The samples were inoculated into the cells for 1 h at 37°C and 5% CO_2 and fresh culture medium (Eagle›s minimum essential medium (EMEM) supplemented with 2% fetal bovine serum (FBS), 0.6 µg/mL penicillin, 60 µg/mL streptomycin, 2 mM L-glutamine, 20 mM HEPES) were added for incubation. On the 4th day of incubation, half of the cultures were blind-passaged onto fresh Vero E6 cells and the rest of original passages were incubated further. After 4 days incubation a clear CPE was detected in the NPA-originated passage 2. The propagation of stock virus was done by passaging a low virus dose once again in Vero E6 cells, and virus culture was harvested on the 3rd day. Virus concentration was followed by RT-PCR. The Ct value for virus passage 1 on the 6th day of incubation was 17.65 and for passage 2 on the 2nd day, before any CPE was 20.63, whereas those of the NPS specimen remained at Ct values between 35 and 36.

SARS-CoV-2/Finland/1/2020 whole-genome sequencing

Nearly the complete coding region of SARS-CoV-2 (GenBank accession number: MT020781) was sequenced from the NPS collected on Day 4 (Table) and the complete coding region was sequenced from the virus isolate obtained after three passages in Vero E6 cells. The virus had 1 nt substitution C21707T compared with the reference strain Wuhan-Hu-1 collected in Wuhan China, December 2019 (NC_045512) [3] which had led to a histidine to tyrosine (H49Y) substitution in the N-terminal domain of the spike glycoprotein.

Antibody response during the SARS-CoV-2 infection

Serum samples were collected from the index case on Days 4, 9, 10 and 20 from onset of the first symptoms (Figure 1). Presence of serum IgM and IgG antibodies against SARS-CoV-2 was analysed by immunofluorescence assays (IFA) based on Vero E6 cells infected with passage 4 of the patient's isolate SARS-CoV-2/Finland/1/2020 virus and transferred onto microscope slides and fixed with acetone (Figure 2). Serum samples from the index case were serially diluted and incubated for 2 h for IgM and 30 min for IgG. Antibodies were visualised with fluorescein isothiocyanate (FITC)-conjugated anti-human IgM or IgG antibodies. While the antibodies were undetectable on Day 4 after onset of symptoms, IgG titres rose to 80 and 1,280 and IgM titres to 80 and 320 on Days 9 and 20, respectively (Table). Random serum samples from staff members of the University of Helsinki (n=19) did not show specific binding at dilutions greater than 20 (Figure 2).

Mock- and SARS-CoV2-infected Vero E6 cells collected on Day 6 post infection were lysed in Laemmli sample

Table

Laboratory data of the first case of SARS-CoV-2 infection, Finland, January–February 2020

Sampling day Day since the first symptoms	Specimen	PCR done at	E	RdRp	N	MN	IgM	IgG
28 Jan 2020 Day 3	NPS	HUS	ND	ND	ND	NA	NA	NA
		THL	30.49	30.48	31.59			
29 Jan 2020 Day 4	NPA	HUS	31.18	27.56	28.29	NA	NA	NA
		THL	27.13	28.43	28.73			
	NPS	HUS	28.15	27.13	28.82	NA	NA	NA
		THL	29.59	30.87	31.78			
	Serum	THL	Neg	Neg	Neg	<4	<20	<20
		UH	Neg	Neg	Neg			
03 Feb 2020 Day 9	NPS	HUS	Neg	Neg	Neg	NA	NA	NA
		THL	Neg	Neg	Neg			
	Serum	UH	ND	Neg	Neg	60	80	80
04 Feb 2020 Day 10	NPS	HUS	Neg	Neg	Neg	NA	NA	NA
		THL	Neg	Neg	Neg			
	Serum	ND	ND	ND	ND	72	160	160
14 Feb 2020 Day 20	Serum	UH	Neg	Neg	Neg	160	320	1,280
17 Feb 2020 Day 23	NPS	HUS	Neg	Neg	Neg	NA	NA	NA
		THL	Neg	Neg	Neg			

E: envelope protein gene; HUS: Helsinki University Hospital Laboratory; IgG: immunoglobulin G; IgM: immunoglobulin M; MN: microneutralisation test; N: nucleocapsid protein gene; NA: not applicable; ND: not done; Neg: negative; NPA: nasopharyngeal aspirate; NPS: nasopharyngeal swab; RdRp: RNA-dependent RNA polymerase gene; RT-PCR: reverse-transcription PCR; THL: Finnish Institute for Health and Welfare; UH: University of Helsinki.

buffer, and Western blotting (WB) of lysates was performed as described previously [4]. At 1:200 dilution, the convalescent serum on Day 20 identified SARS-CoV2 N, S and E protein bands (Figure 3). At higher exposure, all bands were detectable even at 1:1,600 serum dilution (Figure 3).

SARS-CoV-2-specific neutralising antibody levels were measured in duplicate with the MN test in a BSL-3 laboratory. The serum samples were heat-inactivated at 56 °C for 30 min and 2-fold serially diluted starting from 1:4 in EMEM supplemented with 2% of heat-inactivated FBS and antibiotics. Fifty plaque□forming units (PFU) of the SARS-CoV-2/Finland/1/2020 strain were added to the serum dilutions and incubated for 1 h at 37 °C. Vero E6 cells (5×10^4/well) were added to the virus–serum mix, and the mixture was incubated in 96-well plates for 4 days at 37 °C with 5% CO_2. Neutralisation was assessed by CPE. The neutralisation endpoint was determined as the 50% endpoint of the serum that inhibited the SARS-CoV-2 infection observed by CPE of inoculated cells.

Diagnostic serum samples from the index case and her three asymptomatic close contacts were studied with the MN test. During the acute phase of infection, no neutralising antibodies were detected. The patient seroconverted for neutralising antibodies between Day 4 and 9, with the titre increasing to 160 on Day 20 (Table). The serum specimens were confirmed not to be toxic or infective to the cells as such.

Serum samples taken from the three close contacts tested negative in MN test. We also tested serum samples collected in 2019 from 83 Finnish subjects aged 4 to 89 years and all tested negative. Sera known to be positive for IgG against human coronavirus OC43 and 229E [5] and rabbit or guinea pig antibody against SARS-CoV N protein [6] could not neutralise the virus.

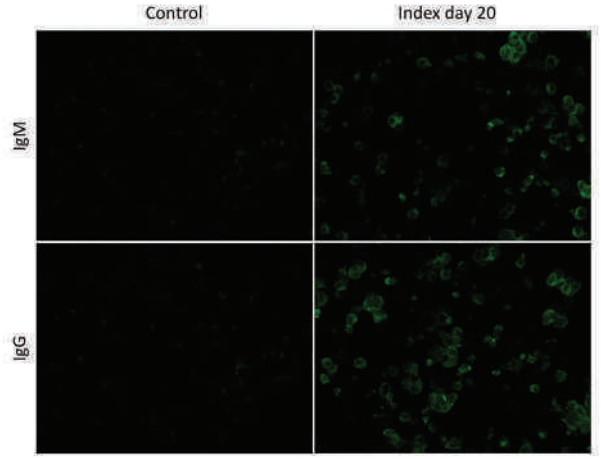
Ethical statement

The investigations were carried out in accordance with the General Data Protection Regulation (Regulation (EU) 2016/679 and Directive 95/46/EC) and the Finnish Personal Data Act (Finlex 523/1999) The Finnish Communicable Diseases Act (Finlex 1227/2016) allows sampling for diagnostic and surveillance purposes.

The convalescent serum sample was obtained on 14 February through informed consent of the patient and research permits (TYH2018322, TYH2019263) from the Helsinki University Hospital Laboratory.
Finnish population serum samples were collected during 2019. The study protocol was approved by the Ethics Committee of the Department of Medicine, Helsinki University Hospital (Permission 433/13/03/00/15).

Figure 2

Immunofluorescence assay of serum samples, COVID-19 index case, Finland, January–February 2020



COVID-19: coronavirus disease 2019.

Anti-SARS-CoV-2 IgM and IgG antibodies were detectable by immunofluorescence assay in samples from Days 9, 10 and 20 after onset of illness. Both IgM and IgG were found at a titre of 80 on Day 9, titres on Day 20 were 320 and 1,280. As an example, dilutions 1:20 and 1:160 from the Day 20 sample are shown for, respectively, IgM and IgG of the index case. Dilution 20 shown for the control serum.

Serum samples of University of Helsinki staff members were used under informed consent.

Discussion

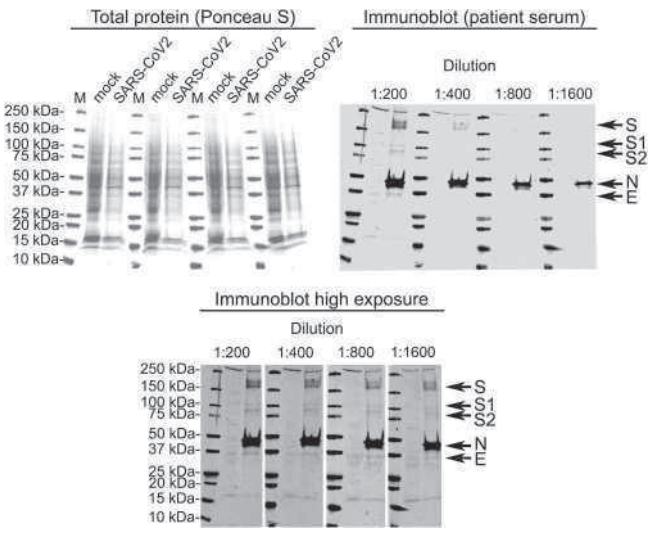
In the early phase of the COVID-19 outbreak, confirmed cases outside China were mostly imported among travellers from Wuhan [7]. The first case in Finland was detected on 29 January among the first imported cases in Europe. The case presented mild symptoms without pneumonia: runny nose, nausea, high fever, cough, muscular weakness and fatigue. No secondary transmission events were detected despite active follow-up by the Lapland Hospital district and THL.

As at 17 March 2020, 358 additional laboratory-confirmed cases of COVID-19 have been detected in Finland. Many of them are travel-related (mostly from northern Italy and Austria) but there is also local transmission from the travel-related cases. The risk of widespread national community transmission of COVID-19 infection in the European Union, European Economic Area and the United Kingdom in the coming weeks is considered high by the European Centre for Disease Prevention and Control [8].

The sequence of the viral genome of the patient was nearly identical to the reference strain from Wuhan, reflecting an early importation from China. Later sequence information in Finland (up to 2 March) showed clustering with strains circulating in Italy (see nextstrain.org/ncov) [9].

Figure 3

Western blot of mock- and SARS-CoV-2 infected Vero E6 cells using patient serum collected 20 days after onset of symptoms, Finland, January–February 2020



Top left panel: total protein staining (Ponceau S) of the nitrocellulose membrane before probing. Top right panel: strips probed with different dilutions of the patient serum at low exposure. Bottom panel: the same membranes individually contrasted for higher band intensity. The arrows indicate SARS-CoV-2 proteins, the labelling assumes that the migration of SARS-CoV-2 proteins was similar to that of Vero E6-expressed SARS-CoV proteins [23]. The bands migrating at ca 110 and 90 kDa probably represent S1 and S2, respectively. Marker M: Precision Plus Dual Colour Standards (Bio-Rad). The detection was done using Odyssey Infrared Imaging System (LI-COR) using goat anti-human IR800 conjugate at 1:10,000 dilution.

Current guidelines from the World Health Organization for testing COVID-19 recommend collection of both acute and convalescent serum samples from patients for serological testing, which can support the identification of the immune response to a specific viral pathogen [10]. The SARS-CoV-2 nucleic acid has been found also in anal swabs and blood [11], however we did not detect it in serum samples in this case. As yet, only limited data are available on antibody responses during SARS-CoV-2 infection [11,12]. Further studies are needed to better understand the seroprevalence of antibodies to different corona viruses in populations and the role of these antibodies in the risk of disease. In accordance with earlier findings [11], we found that both IgM and IgG titres were low or undetectable at on Day 4 (the second day after admission to hospital) yet increasing on Day 9–10, i.e. 5–6 days after the first sampling. Using other detection methods beyond IFA as well as recombinant antigens and analysing samples from a larger number of patients will shed more light on this. The time of first appearance of anti-SARS-CoV antibodies has ranged from Day 3 to 42 and Day 5 to 47 for IgM and IgG antibodies, respectively [13].

The WB of the serum sample collected at convalescence showed a prominent response against the N and S protein, confirming their role as main candidate

diagnostic targets for antibody tests. However, the patient serum appeared to recognise also the E protein and the processed S1 and S2 proteins. Although WB detects mainly linear epitopes, the strong antibody response against the S protein correlated well with the results of the MN assay.

Monitoring of the binding antibodies is suggested to be a more sensitive method than measuring functional neutralising antibodies for serological detection of human coronavirus (hCoV) infections [14]. However, hCoV OC43 and 229E samples can also cross-react with SARS-CoV ELISA testing [15]. The SARS-CoV-2 CPE-based MN test using live virus appeared to be very specific, while laborious to conduct requiring a BSL-3 laboratory. An increase of at least 4-fold in the neutralising antibodies indicating a positive response was detected at Day 9–10 after the first symptoms and at Day 20, the antibody levels were still increasing. Our findings indicate that the MN assay is specific for functional SARS-CoV-2 antibodies and could be applied in surveillance of population immunity for this virus. The assay can be used as confirmatory tool for SARS-CoV-2 specificity in the development of more accessible diagnostic tools such as assays based on detecting binding antibodies. Previous studies on patients with SARS-CoV infection indicated that the median time for seroconversion was 20 days, by which time 60–75% of patients had IgG against the virus [13,16]. That IgM and IgG antibodies were present within 2 weeks from the onset of symptoms in our study suggests that early convalescent patients may be suitable sources of therapeutic antibodies [17]. In accordance with our finding, a recent preprint report on patients admitted to hospital with confirmed SARS-CoV-2 infection in China indicated that the median time to seroconversion was 11–14 days, depending on the immunological assay used [18].

No neutralising SARS-CoV-2 antibodies were detected in the close contacts nor in the control population samples collected during 2019 in Finland. A low prevalence (0.21%) of antibodies against Middle East respiratory syndrome coronavirus was reported in the general population of Qatar [19]. A meta-analysis of seroprevalence to SARS-CoV among different human populations yielded an overall low seroprevalence (0.10%), although it was slightly higher (0.23%) among healthcare workers and others who had close contact with SARS patients [20]. Binding and neutralising HCoV antibodies were found to be higher in older adults [14]. In total 97% and 99% of serum samples from healthy adults had antibodies to HCoV-229E and HCoV-OC43, respectively [21], and 75% and 65% of the children in the age group 2.5–3.5 years were found to be seropositive for, respectively, HCoV-NL63 and HCoV-229E [22]. While it has been suggested that the late seroconversion in most SARS patients reduces the value of serological assays during the incubation and initial phases of SARS [13], serological testing is suggested for the confirmation of a SARS CoV-2 infection [11]. After understanding better the kinetics, specificity and sensitivity of the assays in development, the serological testing may help contact tracing of clusters and have a role in diagnosing acute and past SARS-CoV-2 infections.

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Conflict of interest

LS is a co-investigator in an unrelated study, for which THL has received research funding from GlaxoSmithKline Vaccines. The other authors report no potential conflicts of interest.

Authors' contributions

All authors attest they meet the ICMJE criteria for authorship. All authors have contributed to, seen and approved the final version of the manuscript.

AH set up and performed MN tests, participated in laboratory confirmation of COVID-19 suspicions, coordinated and participated in the collections of the Finnish population sera, and wrote the manuscript.

TS performed the whole genome sequencing and genetic characterisation.

SK and JH performed the IFA and WB analysis, respectively.

PÖ isolated the virus and was responsible of the SARS-CoV-2 related biosafety level 3 laboratory work at THL.

MP, MS, TP and JS participated in THL COVID-19 situation monitoring group work of THL.

SB and MM participated in serological analysis planning group of THL. SB contributed in the interpretation of MN results.

ER set up the real-time RT-PCR method at THL and participated in laboratory confirmation of COVID-19 suspicions.

AK and TS participated in collection of the convalescent serum. AK had a significant role to organising convalescent serum sampling.

HKK, LM and ML were in charge of primary diagnostic of COVID-19 suspicions in HUSLAB. HKK and LM also set up

the real-time RT-PCR method at HUSLAB and participated in laboratory sample logistics from HUSLAB to THL.

MB was responsible for the care of the patient in hospital.

MJ and LS participated in interviewing the index case and contact tracing.

OV was responsible for virological and serological studies in HUSLAB and University of Helsinki, participated in the designing of the study and organising convalescent serum sampling.

NI and CSK were responsible of THL laboratory confirmation of COVID-19 suspicions, participated in COVID-19 situation monitoring group work of THL and laboratory sample logistics. CSK was also responsible for the collections of the Finnish population sera and participated in the designing of the study.

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Instructions for notification of alleged RCR violations

In Finland, alleged research misconduct and other violations of the responsible conduct of research (RCR) are investigated in accordance with the guidelines of the Finnish National Board on Research Integrity TENK *Responsible conduct of research and procedures for handling allegations of misconduct in Finland* (RCR 2012).

The guidelines state that allegations of violation of the responsible conduct of research may be notified on the following terms:

- The notification is to be sent to the organisation in which the research concerned is primarily being conducted/was primarily conducted or in which the researcher concerned was working at the time of the alleged violation.
- Violations of RCR may only be notified to organisations that have committed to follow the RCR guidelines, see the list of organisations on TENK's website.
- Notification must be sent directly to the highest authority at the organisation (e.g. the rector of a university).
- The person making the allegation does not need to be a researcher or a member of the research community.
- Notification may not be anonymous. In problematic situations, the person making the allegation may contact TENK's Secretary General in advance, see contact information on TENK's website.
- Making unfounded and malicious allegations of an RCR violation may in itself be an RCR violation.

Researchers may discuss suspicions of RCR violations in confidence with the Research Integrity Adviser at their own organisation. However, Research Integrity Adviser may not participate in the processing of allegations of RCR violations.

Notification may be made on this form. **The notification is to be sent directly to the rector/head of the organisation concerned.** The contact details of the rector/head of the organisation will be found on the organisation website.

The organisation receiving written notification of an alleged RCR violation sends this notification and the decisions reached in the case, with appended documentation, to TENK and the Research Integrity Adviser in their own organisation for information. Summaries of RCR violations identified in the RCR investigation process are published on TENK's website. TENK does not publish the names of the individuals concerned or the organisations which handled the case.

TENK's actions are guided by the Act on the Openness of Government Activities (1999/621). This being the case, anyone as a rule has the right to receive information about documents in the RCR process sent to TENK where these do not contain information that is to be kept secret (e.g. health data or business secrets).

Notification form

1. Contact details of the person/people submitting the notification

**) compulsory information*

An alleged RCR violation may be notified by one or more people. Where necessary, the details of other people submitting the notification may be given in section 9. Additional information.

Name* [redacted]	E-mail address* [redacted]
Address	
Post code and town	Phone number
Name [redacted]	E-mail address [redacted]
Name	E-mail address

2. Details of the person/people suspected of an RCR violation

An alleged RCR violation may concern more than one person. Where necessary, you may provide the details of other people suspected in section 4. Course of events.

Name Anu Haveri	E-mail address or other contact details (if known) anu.haveri@thl.fi
Title or position Researcher	Organisation THL
Name	E-mail address or other contact details (if known)
Title or position	Organisation
Name	E-mail address or other contact details (if known)
Title or position	Organisation

3. What violation of the responsible conduct of research (RCR) does the allegation primarily concern?

Please choose only one option. Definitions of the RCR violation categories are provided in *Responsible conduct of research and procedures for handling allegations of misconduct in Finland*, the RCR 2012 guidelines (pp. 32–33).

- ☐ fabrication
- ☐ falsification of observations
- ☐ plagiarism or misappropriation
- ☐ violation of authorship
- ☐ other negligence/misleading the research community
- ☐ exaggerating a CV
- ☐ inappropriately hampering the work of another researcher
- ☒ other, please state: scientific misleading and fraud

4. Course of events or description of alleged RCR violation

State briefly what the issue concerns. Additional details such as key evidence material regarding the case may be appended where necessary.

1. **On 6 September 2021, I made a request for information to THL in accordance with the Act on the Openness of Government Use. I asked for evidence of the complete isolation of the coronavirus (Sars-Cov-2) for viewing and public publication, so that the virus is isolated from everything else, as well as evidence of the virus's involvement in the symptoms, in addition to a photograph (note "photo" not "image" of the virus).**
2. **Thl responded to the request two days late on 22 September 2021, claiming as evidence a study carried out in Finland and a few links that can be found in the appendix file. [Annex 1]**
3. **They also claimed in their message that: "The isolation of the virus is talked about when a patient sample is implanted in a cell culture and the virus begins to multiply in it."**
4. **I replied by e-mail that the material they provided would not respond to my request.**
5. **The e-mail chain and other materials can be found in the attachments.**
6. **In addition to the person responsible for the investigation, Anu Haver, this suspicion of offence includes: Teemu Smura, Suvi Kuivanen, Pamela Österlund, Jussi Hepojoki, Niina Ikonen, Marjaana Pitkäpaasi, Soile Blomqvist, Esa Rönkkö, Anu Kantele, Tomas Strandin, Hannimari Kallio-Kokko, Laura Mannonen, Maija Lappalainen, Markku Broas, Miao Jiang, Lotta Siira, Mika Salminen, Taneli Puumalainen, Jussi Sane, Merit Melin, Olli Vapalahti, Carita Savolainen-Kopra**
7. **In addition, the National Institute for Health and Welfare for maintaining incorrect information and not correcting it.**

5. In which publication(s) did the alleged RCR violation occur or in which other context did the alleged violation became apparent?

Bibliographic details of the publication or description of other context. In cases of suspected plagiarism, show the text plagiarised.

Study: "Serological and molecular findings during SARS-Cov-2 infection: the first case study in Finland, January to February 2020" [Annex 2]

6. When did the alleged RCR violation take place?

Date or period of time in which the alleged RCR violation took place.

January to February 2020.

7. Grounds for the allegation

State here the reason why the course of events described above fulfils the criteria for an RCR violation. Use the guidelines *Responsible conduct of research and procedures for handling allegations of misconduct in Finland* to help you and refer to the applicable parts of the guidelines.

In response to the request for information, THL used the study in section 5 to prove the isolation, existence and inclusion of sars-cov-2 virus in the symptoms of a supposed coronavirus patient. However, the study does not prove that the coronavirus in question exists, nor that it causes symptoms. The study explains how to mix a patient's nasopharyndus sample with a cell culture with e.g. vero e6 cells (monkey kidney cells), penicillin (antibiotic), streptomycin (an antibiotic, which is toxic to the kidneys!) and l-glutamine (bovine fetal serum). In addition, the study used a PCR test to show patients had a "COVID-19 infection."

The authors and the THL in their response claim that the patient's sample is mixed with a cell culture as evidence of the virus, as described above. However, it is not a question of 'virus isolation' because

1. **The research method itself causes the destruction of the above cells and tissues used in cell culture, NOT the 'supposedly infected material'.**
2. **Virologists in this case, too, have flouted the basic rules of scientific work and have not carried out CONTROL tests.**
3. **Control tests show that the cells and tissues used in cell culture are completely degraded in the same way, even if the supposedly infected material is not added to the cell culture from patient samples.**
4. **Virologists compile a model of a virus that actually does not exist from short fragments (fragments) of scattered tissues and cells.**
5. **In a 2017 judgment of the German Supreme Court, the entire basis of virology was overturned in the so-called "measles virus trial". The court-appointed expert issued a statement indicating that the cell culture method used since 1954 to isolate the "virus" is not really proof that the "virus" exists. Molecular and marine biologist Tri. Stefan Lanka thus won a trial based on his €100,000 prize on whoever would prove the existence of the measles virus.**

The PCR tests used (and so on antibody tests) are therefore not indicative of any infection or virus, or part of the virus. Genetic virus tests (PCR) show only the body's own sequences (severity of the gene ring). Since the test only shows 'positive' when there are sufficient genetic specimens in the test sample, it is clear why there are also negative test results. Of course, it is clear that, especially in inflammatory events, the body releases more tissue material and with it genetic severities than in a healthy state or when the body at certain moments of healing does not release them at all. All you have to do is increase the amount of test sample (no matter what kind: a swipe sample, blood, mucus, semen, tissue sample, etc.) and so gets every human, every animal and probably even every plant a positive test result.
A more detailed written explanation of the explanatory statement can be found in the Annex [Annex 3]; "Statement on the isolation of the virus". We call on honest scientists, bioinformaticists and laborers to finally conduct and publish those control experiments that have never been conducted or published. We call for the suspicion of injury to be dealt with and for a response as a matter of emergency, because fraudulent virologists are to blame for the coronavirus crisis because they claim (intentionally or deliberately) to isolate viruses using a technique that is already a completely ridiculous and scientific fraud, even by layman's logic.
We also recommend watching a video of the link in the attachments with Tri. Stefan Lanka with his research on 21 April 2021, has refuted the entire fraudulent virology, which unfortunately is also represented by a research group set up by the National Institute for Health and Welfare. The fourth annex is the evidence summary "There are no viruses" by Vesa-Ilkka Laurio (retired MD). You might want to look into it very carefully. There are a lot of Dr. Stefan Lanka's clarifications on the deceitfulness of virology and also the measles virus trial we mentioned, which he won. We are happy to answer your questions if you need further clarification or additional information.

8. Handling of the matter by other organisations

State here if RCR notifications regarding the matter have been made in other research organisations and/or complaints have been made to other bodies (e.g. Parliamentary Ombudsman, Council for Mass Media, Administrative Court), the stage of processing that the case has reached and/or decisions made on the matter by other organisations.

Enter text by clicking or tapping here.

9. Additional information

Here you may state, for example, the details of other parties in the case or associated with the case.

Enter text by clicking or tapping here.

10. List of annexes

Material central to the case can be appended. Annexes must be numbered and must clearly support the alleged RCR violation reported above. The organisation receiving the notification may, where necessary, request additional information from the person making the notification.

Note: Both the RCR notification and the documents appended to it are public where these do not contain confidential data.

Enter text by clicking or tapping here.

11. Date and signature

Date	Person submitting the notification **First name Last name**	Title/profession (not compulsory) **Title or profession**

Terhi Kilpi

5.11.2021

[redacted]

Asia: Hyvän tieteellisen käytännön loukkausepäilyä koskeva ilmoituksenne 13.10.2021

Hyvät vastaanottajat

THL kiittää tutkijoittemme työtä kohtaan osoittamastanne kiinnostuksesta ja toteaa sen johdosta seuraavaa.

Ilmoituksenne kohdistuu tieteelliseen julkaisuun:
Haveri A, Smura T, Kuivanen S, Österlund P, Hepojoki J, Ikonen N, Pitkäpaasi M, Blomqvist S, Rönkkö E, Kantele A, Strandin T, Kallio-Kokko H, Mannonen L, Lappalainen M, Broas M, Jiang M, Siira L, Salminen M, Puumalainen T, Sane J, Melin M, Vapalahti O, Savolainen-Kopra C. Serological and molecular findings during SARS-CoV-2 infection: the first case study in Finland, January to February 2020. Euro Surveill. 2020 Mar;25(11):2000266. doi: 10.2807/1560-7917.ES.2020.25.11.2000266. Artikkelin kirjoittajat edustavat THL:n lisäksi Helsingin ja Zürichin yliopistoja, Helsingin yliopistollista sairaalaa (HUSLAB) ja Lapin keskussairaalaa.

Ilmoitatte epäilevänne artikkelin kirjoittajien syyllistyneen tieteelliseen harhaanjohtamiseen ja tieteelliseen petokseen. Ilmoitukseenne ja sen liitteisiin perehdyttyään THL toteaa, että tieteellistä harhaanjohtamista ja petosta koskeva epäilynne kohdistuu mikrobiologian koko tieteenhaaraan eikä tähän yksittäiseen tutkimukseen. Pääväittämänne, että SARS-CoV-2 -virusta ja viruksia ylipäänsä ei ole ollenkaan olemassa, poikkeaa radikaalisti tiedeyhteisössä laajasti hyväksytystä näkemyksestä. Tutkijaryhmän artikkeli on vertaisarvioitu, mikä tarkoittaa, että ainakin yksi riippumaton asiantuntija on tarkastanut kahdessa eri laboratoriossa viruksen osoittamiseksi tehdyt analyysit ja hyväksynyt viruksen osoitukseen käytetyt menetelmät ja tehdyt johtopäätökset.

Tutkimuseettinen ohjeistus *"Hyvä tieteellinen käytäntö ja sen loukkausepäilyjen käsitteleminen Suomessa"* (HTK-ohje) perustuu Tutkimuseettisen neuvottelukunnan ja tiedeyhteisön yhteistyönä laatimaan ohjeeseen, ja sitä noudattavat keskeiset tieteentekijät, myös THL. HTK-ohjeen mukaan *"[h]yvän tieteellisen käytännön loukkauksilla tarkoitetaan epäeettistä ja epärehellistä toimintaa, joka vahingoittaa tieteellistä tutkimusta ja pahimmillaan mitätöi sen tulokset"*. THL toteaa ettei ilmoituksessanne tai sen liitteissä ole mainintaa sellaisesta menettelystä, joka antaisi aiheen epäillä, että HTK-ohjeen mukaisia hyvän tieteen käytäntöjä ja keskeisiä lähtökohtia olisi loukattu.

Edellä esitetyn perusteella THL katsoo, että ilmoitettu loukkausepäily ei kuulu HTK-ohjeen soveltamisalaan, vaan kyse on muun tyyppisestä ongelmasta. THL ei näin ollen pidä esiselvityksen käynnistämistä aiheellisena.

Ystävällisin terveisin

Markku Tervahauta
Pääjohtaja

Terhi Kilpi
TKI-ylijohtaja

AUTO-TRANSLATION

5.11.2021

[redacted]

[redacted]

Subject: Report of suspected infringement of good scientific practice on 13.10.2021

Good recipients,

THL would like to thank and note the interest shown by our researchers in the work

next.

Your announcement is about a scientific study:

Haveri A, Smura T, Kuivanen S, österlund P, Hepojoki J, Ikonen N, Pitkäpaasi M, Blomqvist S,
Rönkkö E, Kantele A, Strandin T, Kallio-Kokko H, Mannonen L, Lappalainen M, Broas M, Jiang M, Siira
L, Salminen M, Puumalainen T, Sane J, Melin M, Vapalahti O, Savolainen-Kopra C. Serological and
molecular findings during SARS-CoV-2 infection: the first case study in Finland, January to February

2020. Euro Surveil[. 2020 Mar;25(11):2000266. doi:70.280711560-7917.ES.2020.25.17.2000266.
Artikketin kirjoittajat edustavat THL:n lisäksi Hetsingin ja Zürichin yliopistoja, Helsingin yliopistollista sairaalaa (HUSLAB) ja Lapin keskussairaalaa.

You suspect that the authors of the article are guilty of misleading and scientific fraud. After reviewing your report and its attachments, THL will state that the suspicion of deception and fraud is directed at the whole branch of microbiology and not for this single study. Your main claim is that the SARS-CoV-2 virus and viruses It does not exist at all, radically differs from what is widely accepted in the scientific community view. Peer-reviewed article from the research team, meaning that at least one has been tested by an independent expert in two different laboratories for the detection of the virus analyzes and accepted the methods used to detect the virus and the conclusions reached.

Research ethics guidelines "Good scientific practice already in the treatment of its trapping In Finland "(HTK guidelines) is based on the cooperation between the Research Ethics Advisory Board and the scientific community developed by key scientists, including the THL. HTK help according to "[t] he intrusion of scientific practice activity, either by scavenging scientific research and at worst measuring its results. ”THL notes there is no mention in your notice or its annexes of any such procedure suspect that the principles and key principles of good science in accordance with the HTK guideline have been violated.

In view of the above, THL considers that the alleged infringement does not form part of the HTK guidelines but this is another type of problem. THL does not therefore hold a preliminary investigation appropriate.

Regards

Markku Tervahauta
Pääjohtaja

TerhíKitpi

tKl CEO

Online RTI Request Form Details

RTI Request Details :-

RTI Request Registration number	MOHFW/R/E/21/01906
Public Authority	Department of Health & Family Welfare

Personal Details of RTI Applicant:-

Name	[redacted]
Gender	[redacted]
Address	[redacted]
Pincode	[redacted]
Country	India
State	[redacted]
Status	Details not provided
Educational Status	Details not provided
Phone Number	Details not provided
Mobile Number	Details not provided
Email-ID	[redacted]

Request Details :-

Citizenship	Indian
Is the Requester Below Poverty Line ?	Yes
BPL Card No. ***(Proof of BPL may be provided as an attachment)***	Details not provided
Year of Issue	Details not provided
Issuing Authority	Details not provided

(Description of Information sought (upto 500 characters)

Description of Information Sought
see pdf

Concerned CPIO	Nodal Officer
Supporting document ***(only pdf upto 1 MB)***	

Print Close

No. 2

To
Prof. Balram Bhargava
Secretary, DHR & Director General ICMR

With respect to the ongoing health scare, assuming that properly isolated/purified samples of SARS-Cov2 are available, I would like evidence of its causal relationship to a disease.

This should have been carried out by exposing a group of healthy subjects (animals are usually used) to this isolated, purified virus in the manner in which the disease is thought to be transmitted. If the animals got sick with the same disease, as confirmed by clinical and autopsy findings, one has now shown that the virus actually causes a disease. This demonstrates infectivity and transmission of an infectious agent.

This would satisfy one of Germ Theory's important postulates for viruses (Thomas Milton Rivers' postulates/criteria), which is:

The host material with the viral agent used to infect a healthy host (test organism) must be free of any other microorganism and the viral agent obtained from the originally infected host must produce the same specific disease in the suitable healthy host (test organism)

To clarify, I am requesting all such records that are in the possession, custody or control of ICMR or affiliated agencies

Reply by pdf only

सत्यमेव जयते

Select Language: English

Public Authorities Available

RTI Online

Version 2.0

An Initiative of Department of Personnel & Training, Government of India

Online RTI Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	MOHFW/R/E/21/01906
Name	[redacted]
Date of filing	25/04/2021
Public Authority	Department of Health & Family Welfare
Status	REQUEST TRANSFERRED TO OTHER PUBLIC AUTHORITY
Date of action	25/04/2021
Details of Public Autority :- Indian Council of Medical Research. vide registration number :- INCMR/R/T/21/00241 respectively. Note:- Further details will be available on viewing the status of the above-mentioned new request registration number. View Status of INCMR/R/T/21/00241	
Nodal Officer Details :-	
Telephone Number	011-23061831
Email Id	r[dot]attri54[at]nic[dot]in

Print RTI Application Print Status Go Back

सत्यमेव जयते

Select Language: English

Public Authorities Available

RTI Online

Version 2.0

An Initiative of Department of Personnel & Training, Government of India

Online RTI Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	INCMR/R/T/21/00241
Name	[redacted]
Date of filing	25/04/2021
Public Authority	Indian Council of Medical Research
Status	REQUEST DISPOSED OF
Date of action	09/05/2021
Reply :- The study as desired by RTI applicant is not ethical. ICMR does not possess nor conduct any such study	
CPIO Details :-	Dr. Tanu Anand Phone: 9811028964 tanu.anand@icmr.gov.in
First Appellate Authority Details :-	Dr Samiran Panda1 Phone: 011-26588272 samiranpanda.hq@icmr.gov.in
Nodal Officer Details :-	
Telephone Number	011-26588980
Email Id	maheshchand[dot]hq[at]icmr[dot]gov[dot]in

Print RTI Application Print Status Go Back

Online RTI Appeal Form Details

RTI Appeal Details :-

RTI Appeal Registration number	INCMR/A/E/21/00162
Public Authority	Indian Council of Medical Research

Personal Details of Appellant:-

Request Registration Number	INCMR/R/T/21/00241
Request Registration Date	25/04/2021
Name	[redacted]
Gender	[redacted]
Address	[redacted]
Pincode	[redacted]
Country	India
State	[redacted]
Status	Details not provided
Educational Status	Details not provided
Phone Number	Details not provided
Mobile Number	Details not provided
Email-ID	[redacted]

Appeal Details :-

Citizenship	Indian
Is the Requester Below Poverty Line ?	Yes
Ground For Appeal	Refused access to Information Requested
CPIO of Public Authority approached	Dr. Tanu Anand
CPIO's Order/Decision Number	Details not provided
CPIO's Order/Decision Date	

(Description of Information sought (upto 500 characters)

Prayer or Relief Sought	
Please provide scientific proof of contagion/infectiousness.	
Supporting document ***(only pdf upto 1 MB)***	Supporting document not provided

Print Close

सत्यमेव जयते

Select Language: English

Public Authorities Available

RTI Online

Version 2.0

An Initiative of Department of Personnel & Training, Government of India

Online RTI First Appeal Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	INCMR/A/E/21/00162
Name	[redacted]
Date of filing	22/08/2021
Public Authority	Indian Council of Medical Research
Status	COMMENTS SOUGHT FROM CPIO
Date of action	03/09/2021
Appellate Authority Details :- Telephone Number:- 011-26588272, Email Id:- samiranpanda.hq@icmr.gov.in	
First Appellate Authority Details :-	Details not provided
Nodal Officer Details :-	
Telephone Number	011-26588980
Email Id	maheshchand[dot]hq[at]icmr[dot]gov[dot]in

Print RTI First Appeal Print Status Go Back

Online RTI Request Form Details

RTI Request Details :-

RTI Request Registration number	INCMR/R/E/21/00508
Public Authority	Indian Council of Medical Research

Personal Details of RTI Applicant:-

Name	[redacted]
Gender	[redacted]
Address	[redacted]
Pincode	[redacted]
Country	India
State	[redacted]
Status	Rural
Educational Status	Literate
Phone Number	Details not provided
Mobile Number	[redacted]
Email-ID	[redacted]

Request Details :-

Citizenship	Indian
Is the Requester Below Poverty Line ?	No

(Description of Information sought (upto 500 characters)

Description of Information Sought
Request copy of research material that proves that Covid-19 is contagious and spreads from person to person mainly when an infected person breathes out droplets and small particles containing the virus.

Concerned CPIO	Dr Nivedita Gupta
Supporting document ***(only pdf upto 1 MB)***	Supporting document not provided

Print Close

सत्यमेव जयते

Select Language: English

Public Authorities Available

RTI Online

Version 2.0

An Initiative of Department of Personnel & Training, Government of India

Online RTI Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	INCMR/R/E/21/00508
Name	[redacted]
Date of filing	14/06/2021
Public Authority	Indian Council of Medical Research
Status	REQUEST DISPOSED OF
Date of action	21/06/2021
Reply :- The applicant may search published google. There are hundreds of such publications available	
CPIO Details :-	Dr Nivedita Gupta Phone: 011-26588980 ngupta[at]icmr[dot]org[dot]in
First Appellate Authority Details :-	Dr Samiran Panda1 Phone: 011-26588272 samiranpanda[dot]hq[at]icmr[dot]gov[dot]in
Nodal Officer Details :-	
Telephone Number	011-26588980
Email Id	maheshchand[dot]hq[at]icmr[dot]gov[dot]in

Print RTI Application Print Status Go Back

Online RTI Appeal Form Details

RTI Appeal Details :-

RTI Appeal Registration number	INCMR/A/E/21/00110
Public Authority	Indian Council of Medical Research

Personal Details of Appellant:-

Request Registration Number	INCMR/R/E/21/00508
Request Registration Date	14/06/2021
Name	[redacted]
Gender	[redacted]
Address	[redacted]
Pincode	[redacted]
Country	India
State	[redacted]
Status	Rural
Educational Status	Literate
Phone Number	Details not provided
Mobile Number	[redacted]
Email-ID	[redacted]

Appeal Details :-

Citizenship	Indian
Is the Requester Below Poverty Line ?	No
Ground For Appeal	Provided Incomplete,Misleading or False Information
CPIO of Public Authority approached	Dr Nivedita Gupta
CPIO's Order/Decision Number	Details not provided
CPIO's Order/Decision Date	

(Description of Information sought (upto 500 characters)

Prayer or Relief Sought
The lockdowns due to Covid-19 has pushed millions of people into poverty and inconvenienced many people. The reason given for lockdowns was that Covid-19 is contagious and spreads from person to person. In the RTI I had asked ICMR for research material proving that Covid-19 is contagious and spreads from person to person. However the reply given in RTI is that this information can be obtained from google. This is an unsatisfactory response. If ICMR has done its own research on the infectiousness of Covid-19 then it has to share the same. Otherwise it has to given the specific web links that prove that Covid-19 is contagious.

Supporting document ***(only pdf upto 1 MB)***	Supporting document not provided

Print Close

सत्यमेव जयते

Select Language: English

Public Authorities Available

RTI Online

Version 2.0

An Initiative of Department of Personnel & Training, Government of India

Online RTI First Appeal Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	INCMR/A/E/21/00110
Name	[redacted]
Date of filing	21/06/2021
Public Authority	Indian Council of Medical Research
Status	APPEAL DISPOSED OF
Date of action	12/07/2021
Reply :- The reply provided to the applicant is in order and satisfactory. https://www.who.int/news-room/q-a-detail/coronavirus-disease-covid-19-how-is-it-transmitted	
First Appellate Authority Details :-	Dr Samiran Panda1 Phone: 011-26588272 samiranpanda[dot]hq[at]icmr[dot]gov[dot]in
Nodal Officer Details :-	
Telephone Number	011-26588980
Email Id	maheshchand[dot]hq[at]icmr[dot]gov[dot]in

Print RTI First Appeal Print Status Go Back

INCMR/R/E/21/00508 - 2nd appeal filed (Dairy Number is 640247 - filed on 01-Sep-2021)

1. The information requested by the RTI applicant is SCIENTIFIC studies that prove that Covid19 is contagious and spreads from person to person mainly when an infected person breathes out droplets and small particles containing the virus.

2. An RTI application was filed to Indian Councial of Medical Research (ICMR) bearing reference INCMR/R/E/21/00508 dated 14- Jun-2021 seeking this information.

3. ICMR replied on 21-Jun-2021 that this information can be obtained from the internet search engine google, with a comment that there are hundreds of such publications available.

4. Notion of asymptomatic transmission of the virus lead to lockdowns and millions of people were greatly inconvenienced. With this context the response from ICMR is unsatisfactory.

5. First appeal was filed bearing reference INCMR/A/E/21/00110 on 21-Jun-2021.

6. The first appellate authority in the reply dated 12-Jul-2021 directed to the below weblink

https://www.who.int/news-room/q-a-detail/coronavirus-disease-covid19-how-is-it-transmitted

7. This WHO weblink contains already broadcasted information on how Covid-19 spreads. It DOES NOT contain what SCIENTIFIC studies were done to arrive at the conclusion that Covid19 is contagious and spreads from person to person.

8. This second appeal is therefore filed. SCIENTIFIC studies published in scientific journals proving contagiousness of Covid-19 is requested.

Examples of scientific journals are

a. National Library of Medicine - https://pubmed.ncbi.nlm.nih.gov/

b. New England Journal of Medicine - https://www.nejm.org/

c. Cochrane - https://www.cochrane.org/

Hilarious FOI Response From ICMR

[redacted] Sun, Dec 19, 2021 at 9:10 AM

To: Christine Massey <cmssyc@gmail.com>, "christine.massey" <christine.massey@protonmail.com>, christinem <christinem@fluoridefreepeel.ca>

Hi Christine,

We used your FOI text, made some minor changes, explicitly told ICMR what not to send, and these lost souls made us crack up! Their response was downright hilarious.

Please see the attached! This could be a good one for your collection.

Thanks

CantPurifyWithoutIsolating_HumorousNIV_Dec2021ST.pdf
555K

Online RTI Request Form Details

RTI Request Details :-

RTI Request Registration number	NIOVP/R/E/21/00085
Public Authority	ICMR-National Institute of Virology (NIV), Pune

Personal Details of RTI Applicant:-

Name	[redacted]
Gender	[redacted]
Address	[redacted]
Pincode	[redacted]
Country	India
State	[redacted]
Status	Details not provided
Educational Status	Details not provided
Phone Number	Details not provided
Mobile Number	Details not provided
Email-ID	[redacted]

Request Details :-

Citizenship	Indian
Is the Requester Below Poverty Line ?	Yes
BPL Card No. ***(Proof of BPL may be provided as an attachment)***	Details not provided
Year of Issue	Details not provided
Issuing Authority	Details not provided

(Description of Information sought (upto 500 characters)

Description of Information Sought
urgent

Concerned CPIO	Nodal Officer
Supporting document ***(only pdf upto 1 MB)***	

Print Close

Please provide all studies and/or reports in the possession, custody or control of ICMR or NIV or other affiliated bodies/agencies describing the purification of the alleged “COVID-19 virus” (aka “SARS-COV-2”, including any alleged “variants”) directly from a sample taken from a diseased human, where the patient sample was not first combined with any other source of genetic material (i.e. monkey kidney cells aka Vero cells; fetal bovine serum).

Clarification of Request:

Please note that I am NOT requesting studies/reports where researchers failed to purify the suspected “virus” and instead:

1. cultured something, and/or
2. performed an amplification test (i.e. PCR), and/or
3. fabricated a genome from sequences detected in an impure substance, and/or
4. produced electron microscopy images of unpurified things.

I am already aware that according to virus theory a “virus” requires host cells in order to replicate, and am not requesting records that describe replication of a ‘virus’ without host cells. Nor am I requesting records that describe a strict fulfillment of Koch’s Postulates (or Rivers's criteria), or records that describe a suspected “virus” floating in a vacuum, or private patient information.

I am simply requesting records that describe purification (separation of the alleged virus from everything else in the patient sample, as per standard laboratory practices for the purification of other very small things). This would normally involve maceration, filtration, and ultra-centrifugation.

Please note that my request includes any study/report matching the above description, authored by anyone, anywhere.

If any records match the above description of requested records and are currently available in the public domain, please provide enough information about each record so that I may identify and access each one with certainty (i.e. title, author(s), date, journal, where the public may access it). Please provide URLs where possible.

Please do not point me to or send me papers such as the following:

1 Abraham Priya, Cherian Sarah, Potdar Varsha. Genetic characterization of SARS-CoV-2 & implications for epidemiology, diagnostics & vaccines in India. 2020,152 (1), 12-15.

2 Sarkale P, Patil S, Yadav PD, et al. First isolation of SARS-CoV-2 from clinical samples in India. Indian J Med Res. 2020;151(2 & 3):244-250. doi:10.4103/ijmr.IJMR_1029_20.

These are not what I am looking for.

———————————————

Select Language: English

Public Authorities Available

RTI Online

Version 2.0

An Initiative of Department of Personnel & Training, Government of India

Online RTI Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	NIOVP/R/E/21/00085
Name	[redacted]
Date of filing	06/11/2021
Public Authority	ICMR-National Institute of Virology (NIV), Pune
Status	RTI REQUEST APPLICATION RETURNED TO APPLICANT
Date of action	07/12/2021
Reply / Remarks :-Dear Sir, Your RTI application has been replied vide NIV letter No.1/8/2005/RTI/Admn/XVII-2010 dated 07.12.2021. Regards CPIO.	
Nodal Officer Details :-	
Telephone Number	02026006201
Email Id	priya[dot]abraham[at]icmr[dot]gov[dot]in

Print RTI Application Print Status Go Back

आई सी एम आर - राष्ट्रीय विषाणु विज्ञान संस्थान
भारतीय आयुर्विज्ञान अनुसंधान परिषद
स्वास्थ्य अनुसंधान विभाग
स्वास्थ्य एवं परिवार कल्याण मंत्रालय, भारत सरकार

I C M R - NATIONAL INSTITUTE OF VIROLOGY
Indian Council of Medical Research
Department of Health Research
Ministry of Health & Family Welfare, Govt. of India

20 - ए. डा. आंबेडकर मार्ग, पोस्ट बॉक्स सख्या 11, पुणे - 411 001, भारत. 20-A, Dr. Ambedkar Road, Post Box No. 11, Pune 411 001, India.
Tel. : NIV Camp +91-020-26127301, 26006290, Fax : 26122669, 26126643 / NIV Pashan +91-020-26006390 Fax : No. 25871895 / 25870640
E-mail : director.niv@icmr.gov.in Website : www.niv.co.in

No. 1/8/2005/RTI/Admn./XVII- 2009 07th December, 2021

To

[redacted]

Sub.: Online Application under Right to Information Act 2005
Ref.: Registration No. NIOVP/R/E/21/00083 dated 06/11/2021

Madam,

This is in reference to your above online application no. NIOVP/R/E/21/00083 dated 6th November, 2021 seeking information under Right to Information Act 2005. The information sought by you is furnished below.

Please provide all studies and/or reports in the possession, custody or control of ICMR or NIV or other affiliated bodies /agencies describing the purification of the alleged "COVID-19 virus" (aka "SARS-CoV-2", including any alleged "variants") directly from a sample taken from a diseased human, where the patient sample was not first combined with any other source of genetic material (i.e. monkey kidney cells aka Vero cells; Fetal Bovine Serum).	As per our knowledge, there is no methodology available to purify the SARS-CoV-2 directly from the clinical specimens of the patient until the virus is isolated using in vitro or in vivo methods. Considering the biosecurity aspects, the records of the clinical specimens, isolates of SARS-CoV-2 in possession, custody or control of ICMR-National Institute of Virology, Pune cannot be shared publicly.

The Appellate Authority in respect of the information furnished above is, Prof. Priya Abraham, Director, ICMR-National Institute of Virology, Pune. If you are not satisfied with this reply, you may appeal within 30 days of receipt of this letter.

Thanking you,

Yours sincerely,

Deepti Parashar

Dr. Deepti Parashar
CPIO & Scientist-E

Online RTI Request Form Details

RTI Request Details :-

RTI Request Registration number	MOHFW/R/E/21/05264
Public Authority	Department of Health & Family Welfare

Personal Details of RTI Applicant:-

Name	[redacted]
Gender	[redacted]
Address	[redacted]
Pincode	[redacted]
Country	India
State	[redacted]
Status	Details not provided
Educational Status	Details not provided
Phone Number	Details not provided
Mobile Number	Details not provided
Email-ID	[redacted]

Request Details :-

Citizenship	Indian
Is the Requester Below Poverty Line ?	No

(Description of Information sought (upto 500 characters)

Description of Information Sought	
APPLICATION ATTACHED	
Concerned CPIO	Nodal Officer
Supporting document ***(only pdf upto 1 MB)***	

Print Close

6
sub-please provide information under RTI act 2005 and section 76 of Indian evidence act.
"Please see this: https://www.fluoridefreepeel.ca/68-health-science-institutions-globally-all-failed-to-cite-even-1-record-of-sars-cov-2-purification-by-anyone-anywhere-ever/

Here we can see that 93 health/science institutions globally all failed to cite even 1 record of “SARS-COV-2” purification, by anyone, anywhere, ever.

This is for the original so-called SARS-COV2 virus. Given this, please explain the scientific basis for purification and isolation of variants. Does ICMR have any record of this? Please provide scientific papers clearly detailing the purification and isolation of the so-called Delta variant.

By purification and isolation, we mean maceration, filtration, and ultracentrifugation. We do not mean a virus culture created by using a mixture of monkey kidney cells, bovine serum, antibiotics, and so on in a minimal nutrition medium, that too without a CONTROL study.

By doing this it is clear that the process of poisoning the cells (with antibiotics known to be harmful to kidney cells) and starving the cells is what brings about the cytopathic effect (CPE).

सत्यमेव जयते

Select Language: English

Public Authorities Available

RTI Online

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An Initiative of Department of Personnel & Training, Government of India

Online RTI Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	MOHFW/R/E/21/05264
Name	[redacted]
Date of filing	23/08/2021
Public Authority	Department of Health & Family Welfare
Status	REQUEST TRANSFERRED TO OTHER PUBLIC AUTHORITY
Date of action	23/08/2021
Details of Public Autority :- Indian Council of Medical Research. vide registration number :- INCMR/R/T/21/01016 respectively. Note:- Further details will be available on viewing the status of the above-mentioned new request registration number. View Status of INCMR/R/T/21/01016	
Nodal Officer Details :-	
Telephone Number	011-23061831
Email Id	r[dot]attri54[at]nic[dot]in

Print RTI Application Print Status Go Back

सत्यमेव जयते

Select Language: English

Public Authorities Available

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An Initiative of Department of Personnel & Training, Government of India

Online RTI Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	INCMR/R/T/21/01016
Name	[redacted]
Date of filing	23/08/2021
Public Authority	Indian Council of Medical Research
Status	RTI REQUEST APPLICATION RETURNED TO APPLICANT
Date of action	23/08/2021
Reply / Remarks :- COVID-19 related ICMR guidelines, advisories, publications etc, that can be disseminated, are already available at public domain icmr.gov.in. Please follow the ICMR website for updation. Interrogative, personal and hypothetical questions are not covered under the RTI Act 2005. There is no provision under the RTI Act 2005 to seek explanation. Regards.	
Nodal Officer Details :-	
Telephone Number	011-26588980
Email Id	maheshchand[dot]hq[at]icmr[dot]gov[dot]in

Print RTI Application Print Status Go Back

Online RTI Appeal Form Details

RTI Appeal Details :-

RTI Appeal Registration number	INCMR/A/E/21/00167
Public Authority	Indian Council of Medical Research

Personal Details of Appellant:-

Request Registration Number	INCMR/R/T/21/01016
Request Registration Date	23/08/2021
Name	[redacted]
Gender	[redacted]
Address	[redacted]
Pincode	[redacted]
Country	India
State	[redacted]
Status	Details not provided
Educational Status	Details not provided
Phone Number	Details not provided
Mobile Number	Details not provided
Email-ID	[redacted]

Appeal Details :-

Citizenship	Indian
Is the Requester Below Poverty Line ?	No
Ground For Appeal	Provided Incomplete,Misleading or False Information
CPIO of Public Authority approached	Nodal Officer
CPIO's Order/Decision Number	Details not provided
CPIO's Order/Decision Date	

(Description of Information sought (upto 500 characters)

Prayer or Relief Sought
"Please provide scientific papers clearly detailing the purification and isolation of the so-called Delta variant. By purification and isolation, we mean maceration, filtration, and ultracentrifugation. We do not mean a virus culture created by using a mixture of monkey kidney cells, bovine serum, antibiotics, and so on in a minimal nutrition medium"

Supporting document ***(only pdf upto 1 MB)***	Supporting document not provided

Print Close

सत्यमेव जयते

Select Language: English

Public Authorities Available

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An Initiative of Department of Personnel & Training, Government of India

Online RTI First Appeal Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	INCMR/A/E/21/00167
Name	[redacted]
Date of filing	27/08/2021
Public Authority	Indian Council of Medical Research
Status	APPEAL DISPOSED OF
Date of action	30/08/2021
Reply :- I agree with the reply of CPIO ICMR. You can't seek explanation of ICMR on some hypothesis mentioned in your question. This is not covered under the RTI Act 2005. Thanks	
First Appellate Authority Details :-	Dr R Lakshminarayanan Phone: 011-26588980 lakshminarayanan[dot]r[at]icmr[dot]gov[dot]in
Nodal Officer Details :-	
Telephone Number	011-26588980
Email Id	maheshchand[dot]hq[at]icmr[dot]gov[dot]in

Print RTI First Appeal Print Status Go Back

Online RTI Request Form Details

RTI Request Details :-

RTI Request Registration number	INCMR/R/E/21/00768
Public Authority	Indian Council of Medical Research

Personal Details of RTI Applicant:-

Name	[redacted]
Gender	[redacted]
Address	[redacted]
Pincode	[redacted]
Country	India
State	[redacted]
Status	Details not provided
Educational Status	Details not provided
Phone Number	Details not provided
Mobile Number	Details not provided
Email-ID	[redacted]

Request Details :-

Citizenship	Indian
Is the Requester Below Poverty Line ?	No

(Description of Information sought (upto 500 characters)

Description of Information Sought
Please provide scientific papers clearly detailing the purification and isolation of the so-called Delta variant. By purification and isolation, we mean maceration, filtration, and ultracentrifugation. We do not mean a virus culture created by using a mixture of monkey kidney cells, bovine serum, antibiotics, and so on in a minimal nutrition medium.

Concerned CPIO	Nodal Officer
Supporting document ***(only pdf upto 1 MB)***	Supporting document not provided

Print Close

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Select Language: English

Public Authorities Available

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An Initiative of Department of Personnel & Training, Government of India

Online RTI Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	INCMR/R/E/21/00768
Name	[redacted]
Date of filing	08/09/2021
Public Authority	Indian Council of Medical Research
Status	REQUEST TRANSFERRED TO OTHER PUBLIC AUTHORITY
Date of action	08/09/2021
Details of Public Autority :- ICMR-National Institute of Virology (NIV), Pune. vide registration number :- NIOVP/R/T/21/00011 respectively. Note:- Further details will be available on viewing the status of the above-mentioned new request registration number. View Status of NIOVP/R/T/21/00011	
Nodal Officer Details :-	
Telephone Number	011-26588980
Email Id	maheshchand[dot]hq[at]icmr[dot]gov[dot]in

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सत्यमेव जयते

Select Language: English

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Online RTI Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	NIOVP/R/T/21/00011
Name	[redacted]
Date of filing	08/09/2021
Public Authority	ICMR-National Institute of Virology (NIV), Pune
Status	RTI REQUEST APPLICATION RETURNED TO APPLICANT
Date of action	24/09/2021
Reply / Remarks :-	The reply to your application has been sent vide NIV Pune letter no. 1/8/2005/RTI/Admn./XVII-1509 dated 24.09.2021
Nodal Officer Details :-	
Telephone Number	02026006201
Email Id	priya[dot]abraham[at]icmr[dot]gov[dot]in

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आई सी एम आर – राष्ट्रीय विषाणु विज्ञान संस्थान
भारतीय आयुर्विज्ञान अनुसंधान परिषद
स्वास्थ्य अनुसंधान विभाग
स्वास्थ्य एवं परिवार कल्याण मंत्रालय, भारत सरकार
I C M R - NATIONAL INSTITUTE OF VIROLOGY
Indian Council of Medical Research
Department of Health Research
Ministry of Health & Family Welfare, Govt. of India
20 - ए. डा. आंबेडकर मार्ग, पोस्ट बॉक्स संख्या 11, पुणे - 411 001, भारत. 20-A. Dr. Ambedkar Road, Post Box No. 11, Pune 411 001, India.
Tel. : NIV Camp +91-020-26127301, 26006290, Fax : 26122669, 26126643 / NIV Pashan +91-020-26006390 Fax : No. 25871895 / 25870640
E-mail : director.niv@icmr.gov.in Website : www.niv.co.in

No. 1/8/2005/RTI/Admn./XVII- 1509 24th September 2021

To

[redacted]

Sub.: Online Application under Right to Information Act 2005
Ref.: Registration No. NIOVP/R/T/21/00011 dated 08/09/2021

Madam,

This is in reference to your above online application no. NIOVP/R/T/21/00011 dated 8th September, 2021 forwarded by ICMR on 08/09/2021 with reference no. INCMR/R/E/21/00768 seeking information under Right to Information Act 2005. The information sought by you is furnished below.

Please provide scientific papers clearly detailing the purification and isolation of the so-called Delta variant. By purification and isolation, we mean maceration, filtration and ultra-centrifugation. We do not mean a virus culture created by using a mixture of monkey kidney cells, bovine serum, antibiotics, and so on in a minimal nutrition medium.	• There is no specific protocol for purification and isolation of the SARS-CoV-2 Delta variant. Virus purification from plaque is a generalize technique which can be used for various viruses. • The scientific article on SARS-CoV-2 purification and isolation as mentioned below. ➢ Hanifehnezhad A, Kehribar EŞ, Öztop S, Sheraz A, Kasırga S, Ergünay K, Önder S, Yılmaz E, Engin D, Oğuzoğlu TÇ, Şeker UÖ. Characterization of local SARS-CoV-2 isolates and pathogenicity in IFNAR-/-mice. Heliyon. 2020; 6(9):e05116.

The Appellate Authority in respect of the information furnished above is, Prof. Priya Abraham, Director, ICMR-National Institute of Virology, Pune. If you are not satisfied with this reply, you may appeal within 30 days of receipt of this letter.

Thanking you,

Yours sincerely,

Deepti Parashar

Dr. Deepti Parashar
CPIO & Scientist-E

The response provided by the Indian Council of Medical Research (ICMR) did **NOT** correspond to my description of requested records. The ICMR's response consisted of 1 citation, for a study that is **NOT** responsive to my request and that in fact matches my description of the type of study that I was **NOT** requesting.

The study referenced to me in reply: *"First isolation of SARS-CoV-2 from clinical samples in India"* published in the Indian Journal of Medical Research does **NOT** describe the purification of any "SARS COV 2" from a patient sample that was **NOT** first combined with any other source of genetic material (i.e monkey kidney cells aka Vero cells; Fetal bovine serum etc) via maceration, filtration and use of an ultracentrifuge, **which is what I requested**.

This paper also does **NOT** describe the purification of any type of particle, even from a contaminated patient sample. It does **NOT** describe the purification of a suspected virus from any source.

This study does describe exactly what I had explained I was NOT interested in:

- culturing an unpurified substance,
- performing an amplification test (PCR test) on an unpurified substance,
- producing a "genome" of an unpurified substance, and
- producing electron microscopy images of unpurified things.

Below are excerpts from the study which was referenced by ICMR in their reply:

QUOTE

We describe here the successful isolation and characterization of SARS-CoV-2 from clinical samples in India using Vero CCL-81 cells by observing cytopathic effects (CPEs) and cycle threshold (Ct) values in real-time reverse transcription-polymerase
chain reaction (RT-PCR), electron microscopy and next-generation sequencing (NGS)...

...The clinical specimens [not purified "SARS-COV-2"] of the 12 cases were used for infecting Vero CCL-81 which was maintained in Eagle's minimum essential medium (MEM; Gibco, UK) supplemented with 10 per cent foetal bovine serum (FBS) (HiMedia, Mumbai), penicillin (100 U/ml) and streptomycin (100 mg/ml). Likewise, 100 µl was inoculated onto 24-well cell culture monolayers of Vero CCL-81, before the growth medium was decanted...

...From each well of cell culture plate, on the third post-infection day (PID-3) of passage-1 (P-1), 50 µl of supernatant [not purified "SARS-COV-2"] was taken and tested for SARS-CoV-2 using real-time RT-PCR...

...Next-generation sequencing was performed on SARS-CoV-2 positive clinical samples [not purified "SARS-COV-2"] (100 µl) included in the study and the tissue culture fluid [not purified "SARS-COV-2"] (50 µl) of virus isolates at PID-3 as described earlier...

... an aliquot of cell culture supernatant [not purified "SARS-COV-2"] was harvested from infected Vero CCL-81 showing CPE and the supernatant used for negative staining as described elsewhere. Distinct CoV particles with an average size of 95±10 nm having a distinct envelope fringe could be detected in the fields scanned (Fig. 3), as observed earlier.

UNQUOTE

Also I am attaching my RTI request again and I hope a proper reply which is NOT misleading and false information to the request below is given with due diligence:

> This is my formal request for access to general records, made under Right To Information Act.
> Description of Requested records:
> All the studies and/or reports in the possession, custody or control of Indian Council of Medical Research (ICMR) describing the purification of any "SARS COV 2" aka "Covid 19 virus" (including any "variants") (via maceration, filtration and use of an ultracentrifuge; also referred to at times by some people
> as "isolation"), directly from a sample taken from a "diseased" human, where
> the patient sample was not first combined with any other source of genetic material (i.e monkey kidney cells aka Vero cells; Fetal bovine serum etc).
> Please note that I am not requesting studies/reports where the researchers failed
> to purify the suspected "virus" and instead:
> * cultured an unpurified sample or other unpurified substance, and/or
> *performed an amplification test (i.e. a PCR test) on all the RNA from a patient
> sample or from a cell culture, or on genetic material from any unpurified substance, and/or
> *sequenced the total RNA from a patient sample or from a cell culture or from any unpurified substance, and/or
> *produced electron microscopy images of unpurified things.
> Clarification regarding my request:
> For further clarity, please note I am already aware that according to the virus theory a "virus" requires host cells in order to replicate, and I am not requesting
> records describing the replication of a "virus" without host cells.

Further, I am not requesting records that describe a suspected "virus" floating in
a vacuum; I am simply requesting records that describe its purification (separation from everything else in the patient sample, as per standard laboratory practices for purification of other smaller things).
Please also note that my request is not limited to records that were authored by
ICMR or that pertain to work done at/by ICMR and its associate organisations. Rather, my request includes any record matching the above description, for example (but not limited to) any published peer-reviewed study authored by anyone, anywhere that has been downloaded or printed by Administration or Staff at ICMR and relied on as evidence of a disease-causing "virus".
If any records match the above description of requested records and are currently available to the public elsewhere, provide enough information about each record so that I may identify and access each one with certainty (i.e. title, author(s), date, journal, where the public may access it). Please provide URLs where possible.

Select Language: English

Public Authorities Available

RTI Online

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An Initiative of Department of Personnel & Training, Government of India

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Online RTI First Appeal Status Form

Note:Fields marked with * are Mandatory

Enter Registration Number	[redacted]
Name	Yash [redacted]
Date of filing	04/05/2021
Public Authority	Indian Council of Medical Research
Status	APPEAL DISPOSED OF
Date of action	12/07/2021
Reply :- The reply provided to the applicant is in order and satisfactory.	
First Appellate Authority Details :-	Dr Samiran Panda1 Phone: 011-26588272 samiranpanda.hq@icmr.gov.in
Nodal Officer Details :-	
Telephone Number	011-26588980
Email Id	devishant[at]icmr[dot]gov[dot]in

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आई सी एम आर – राष्ट्रीय विषाणु विज्ञान संस्थान
भारतीय आयुर्विज्ञान अनुसंधान परिषद
स्वास्थ्य अनुसंधान विभाग
स्वास्थ्य एवं परिवार कल्याण मंत्रालय, भारत सरकार
I C M R - NATIONAL INSTITUTE OF VIROLOGY
Indian Council of Medical Research
Department of Health Research
Ministry of Health & Family Welfare, Govt. of India
20 - ए. डा. आंबेडकर मार्ग, पोस्ट बॉक्स संख्या 11, पुणे - 411 001, भारत. 20-A. Dr. Ambedkar Road, Post Box No. 11, Pune 411 001, India.
Tel. : NIV Camp +91-020-26127301, 26006290, Fax : 26122669, 26126643 / NIV Pashan +91-020-26006390 Fax : No. 25871895 / 25870640
E-mail : director.niv@icmr.gov.in Website : www.niv.co.in

No. 1/8/2005/RTI/Admn./XVII- 669

28th June 2021

To

Sh. Trinayan Das
Kamarchuburi,
NT Road, Tezpur,
Sonitpur, Assam – 784001

Sub.: Online Application under Right to Information Act 2005
Ref.: Registration No. NIOVP/R/E/21/00038 dated 16/06/2021

Sir,

This is in reference to your above online application no. NIOVP/R/E/21/00038 dated 16th June 2021, seeking information under Right to Information Act 2005. The information sought by you is furnished below.

1. Any proof of isolation/purification of SARS-CoV-2 (COVID-19) virus?	Please find the below mentioned publications for the SARS-CoV-2 isolations by ICMR-National Institute of Virology. a. Sarkale P., Patil, S., Yadav, P.D., Nyayanit, D.A., Sapkal, G., Baradkar, S., Lakra, R., Shete-Aich, A., Prasad, S., Basu, A. and Dar, L., 2020. First isolation of SARS-CoV-2 form clinical samples in India. The *Indian Journal of Medical Research*, 11(2-3), p.244. b. Yadav, P., Sarkale, P., Razdan, A., Gupta, N., Nyayanit, D., Sahay, R., Potdar, V., Patil, D., Baradkar, S., Kumar, A. and Aggarwal, N., 2021. Isolation and characterization of SARS-CoV-2 VOC, 20H/501Y. V2, from UAE travelers, bioRxiv. c. Yadav, P.D., Nyayanit, D.A., Sahay, R.R., Sarkale, P., Pethani, J., Patil, S., Baradkar, S., Potdar, V. and Patil, D.Y., 2021. Isolation and characterization of the new SARS-CoV-2 variant in travelers from the United Kingdom to India: VUI-202012/01 of the B. 1.1. 7 lieage. *Journal of Travel Medicine*, 28(2),

	p.taab009. d. Yadav, P.D., Nyayanit, D.A., Sahay, R.R., Shete, A.M., Majumdar, T., Patil, S., Patil, D.Y., Gupta, N., Kaur, H., Aggarwal, N. and Vijay, N., 2021. Imported SARS-CoV-2 V501Y. V2 variant (B. 1.351) detected in travelers from South Africa and Tanzania to India. *Travel Medicine and Infectious Disease.*
2. What are the methods used for isolation/purification of SARS-CoV-2 virus?	Virus isolation is being performed in Vero cell lines using the tissue culture techniques.
3. Is the RT-PCR test approved for diagnostic of infectious disease like SARS-CoV-2 (COVID-19) virus?	Yes, Real Time Reverse Transcription Polymerase Chain Reaction (Real Time RT-PCR) is the gold standard test for detection of SARS-CoV-2. For more details on molecular testing of SARS-CoV-2 please refer to ICMR advisory available at https://www.icmr.gov.in/pdf/covid/labs/ICMR_Advisory_Testing_System_v_10112020.pdf and the WHO guidelines available at https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf
4. Was the RT-PCR test used earlier to diagnose any infectious disease? What is the accuracy of the test?	Yes, RT-PCR test is a widely used test to detect many infectious diseases such as Influenza viruses, Hepatitis viruses, HIV, Dengue chikungunya etc. it is widely used in biomedical science research. The test is highly sensitive and detects specific targets very accurately.
5. How does the PCR test help in diagnosing SARS-CoV-2 virus genetic sequence?	Real-Time PCR offers sensitivity, specificity and wide dynamic range for detecting target nucleic acids. For the SARS-CoV-2 detection variety of RT-PCR kits are available. The RT-PCR of SARS-CoV-2 detects more than two genes of SARS-CoV-2 such as E, N, ORF, S, RDRP along with human housekeeping genes as sample quality targets. The positive results in RT PCR are confirming the presence of SARS-CoV-2 virus infection.
6. What is the false positive rate of PCR test on CT-35?	The RT-PCR kit has a cut off range to determine positivity in tests. Majority kits have the set Cut off values ranging from 35 to 40 ct value. As mentioned above SARS-CoV-2 kits use multiple targets for detection and the decision of the sample as positive is dependent on the targets used in the test. If a single gene showed ct value 35 then the sample is inconclusive and recommended to

	repeat after four days. The main reason for this kind of report may be improper timing of specimen collection e.g. early phase of infection or recovery phase of infection. Any specimen that has a Ct below the cut off for the test is most likely a true positive. Ct values can differ immensely between a poorly collected specimen to a well-collected specimen. Other factors than can impact Ct values include improper specimen transport, specimen storage temperatures, how many times the specimen has been frozen, and the instrument on which testing is performed. Each test is different, with different sensitivities based on things like how the test was designed.
7. Can a N95 face mask prevent the transmission of SARS-CoV-2 virus?	An N95 mask is a respiratory protective device designed to achieve a very close facial fit and very efficient filtration of airborne particles. N95 masks without gaps can filter 99.9 percent particles larger than 0.3um and 85 percent particles smaller than 0.3um.
8. Any proof of isolation/purification of the Delta variant or any other variants of SARS-CoV-2?	Please find the below mentioned publications for the SARS-CoV-2 isolations by ICMR-National Institute of Virology. a. Sarkale P., Patil, S., Yadav, P.D., Nyayanit, D.A., Sapkal, G., Baradkar, S., Lakra, R., Shete-Aich, A., Prasad, S., Basu, A. and Dar, L., 2020. First isolation of SARS-CoV-2 form clinical samples in India. The *Indian Journal of Medical Research*, 11(2-3), p.244. b. Yadav, P., Sarkale, P., Razdan, A., Gupta, N., Nyayanit, D., Sahay, R., Potdar, V., Patil, D., Baradkar, S., Kumar, A. and Aggarwal, N., 2021. Isolation and characterization of SARS-CoV-2 VOC, 20H/501Y. V2, from UAE travelers, bioRxiv. c. Yadav, P.D., Nyayanit, D.A., Sahay, R.R., Sarkale, P., Pethani, J., Patil, S., Baradkar, S., Potdar, V. and Patil, D.Y., 2021. Isolation and characterization of the new SARS-CoV-2 variant in travelers from the United Kingdom to India: VUI-202012/01 of the B. 1.1. 7 lieage. *Journal of Travel Medicine*, 28(2),

	p.taab009. d. Yadav, P.D., Nyayanit, D.A., Sahay, R.R., Shete, A.M., Majumdar, T., Patil, S., Patil, D.Y., Gupta, N., Kaur, H., Aggarwal, N. and Vijay, N., 2021. Imported SARS-CoV-2 V501Y. V2 variant (B. 1.351) detected in travelers from South Africa and Tanzania to India. *Travel Medicine and Infectious Disease.*
9. Was there any tissue culture done on the SARS-CoV-2 virus?	Yes, virus was cultured for development of indigenous inactivated vaccine and for development of ELISA and neutralization assays.

The Appellate Authority in respect of the information furnished above is, Prof. Priya Abraham, Director, ICMR-National Institute of Virology, Pune. If you are not satisfied with this reply, you may appeal within 30 days of receipt of this letter.

Thanking you,

Yours sincerely,

Dr. Paresh Shah
CPIO & Scientist-E

This is my formal request for access to general records, made under Right To Information Act.

Description of Requested records:

All the studies and/or reports in the possession, custody or control of Indian Council of Medical Research (ICMR) describing the purification of any "SARS COV 2" aka "Covid 19 virus" (including any "variants") (via maceration, filtration and use of an ultracentrifuge; also referred to at times by some people as "isolation"), directly from a sample taken from a "diseased" human, where the patient sample was not first combined with any other source of genetic material (i.e monkey kidney cells aka Vero cells; Fetal bovine serum etc).

Please note that I am not requesting studies/reports where the researchers failed to purify the suspected "virus" and instead:

* cultured an unpurified sample or other unpurified substance, and/or
*performed an amplification test (i.e. a PCR test) on all the RNA from a patient sample or from a cell culture, or on genetic material from any unpurified substance, and/or
*sequenced the total RNA from a patient sample or from a cell culture or from any unpurified substance, and/or
*produced electron microscopy images of unpurified things.

Clarification regarding my request:

For further clarity, please note I am already aware that according to the virus theory a "virus" requires host cells in order to replicate, and I am not requesting records describing the replication of a "virus" without host cells.

Further, I am not requesting records that describe a suspected "virus" floating in a vacuum; I am simply requesting records that describe its purification (separation from everything else in the patient sample, as per standard laboratory practices for purification of other smaller things).

Please also note that my request is not limited to records that were authored by ICMR or that pertain to work done at/by ICMR and its associate organisations.

Rather, my request includes any record matching the above description, for example (but not limited to) any published peer-reviewed study authored by anyone, anywhere that has been downloaded or printed by Administration or Staff at ICMR and relied on as evidence of a disease-causing "virus".

If any records match the above description of requested records and are currently available to the public elsewhere, provide enough information about each record so that I may identify and access each one with certainty (i.e. title, author(s), date, journal, where the public may access it). Please provide URLs where possible.

Select Language: English

Public Authorities Available

RTI Online

Version 2.0
An initiative of Department of Personnel & Training, Government of India

Home Submit Request Submit First Appeal View Status View History New User Manual FAQ

Online RTI Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	[redacted]
Name	[redacted]
Date of filing	25/04/2021
Public Authority	Indian Council of Medical Research
Status	REQUEST DISPOSED OF
Date of action	03/05/2021
Reply :- For all the queries a research article has been published in the year 2020. Ref:- Prasad Sarkale, Savita Patel, Pragya Yadav, et al., First Isolation of SARS-Cov-2 from clinical samples in India, Indian Journal of Medical Research. This article will answer your queries:- SARS-Cov-2 was successfully isolated and characterized from clinical samples in India using Vero CCL-81 cells by observing cytopathic effects (CPE's)and cycle threshold (Ct) values in real-time reverse transcription-polymerase chain reaction (RT-PCR), electron microscopy and next-generation sequencing (NGS).	
CPIO Details :-	Dr Nivedita Gupta Phone: 011-26588980 ngupta@icmr.org.in
First Appellate Authority Details :-	Dr Samiran Panda Phone: 011-26588895 pandas.hq@icmr.gov.in
Nodal Officer Details :-	
Telephone Number	011-26588980
Email id	davishant[at]icmr[dot]gov[dot]in

Print RTI Application Print Status Go Back

Date of filing	25/04/2021
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First Appellate Authority Details :-	Dr Samiran Panda Phone: 011-26588895 pandas.hq@icmr.gov.in
Nodal Officer Details :-	
Telephone Number	011-26588980
Email Id	devishanti[at]icmr[dot]gov[dot]in

आई सी एम आर – राष्ट्रीय विषाणु विज्ञान संस्थान
भारतीय आयुर्विज्ञान अनुसंधान परिषद
स्वास्थ्य अनुसंधान विभाग
स्वास्थ्य एवं परिवार कल्याण मंत्रालय, भारत सरकार
ICMR - NATIONAL INSTITUTE OF VIROLOGY
Indian Council of Medical Research
Department of Health Research
Ministry of Health & Family Welfare, Govt. of India
20 - ए, डा. आंबेडकर मार्ग, पोस्ट बॉक्स संख्या 11, पुणे - 411 001, भारत. 20-A, Dr. Ambedkar Road, Post Box No. 11, Pune 411 001, India.
el. : NIV Camp +91-020-26127301, 26006290, Fax : 26122669, 26126643 / NIV Pashan +91-020-26006390 Fax : No. 25871895 / 25870640
E-mail : director.niv@icmr.gov.in Website : www.niv.co.in

No. 1/8/2005/RTI/Admn./XVII- 671

28th June 2021

To

[redacted]

Sub.: Online Application under Right to Information Act 2005
Ref.: Registration No. NIOVP/R/T/21/00005 dated 12/06/2021

Sir,

This is in reference to your above online application no. NIOVP/R/T/21/00005 dated 12th June 2021 forwarded by ICMR with reference no. INCMR/R/T/21/00577 dated 12/06/2021, seeking information under Right to Information Act 2005. Please find the below mentioned publications for the SARS-CoV-2 isolations by ICMR-National Institute of Virology.

a. Sarkale P., Patil, S., Yadav, P.D., Nyayanit, D.A., Sapkal, G., Baradkar, S., Lakra, R., Shete-Aich, A., Prasad, S., Basu, A. and Dar, L., 2020. First isolation of SARS-CoV-2 form clinical samples in India. The *Indian Journal of Medical Research*, 11(2-3), p.244.
b. Yadav, P., Sarkale, P., Razdan, A., Gupta, N., Nyayanit, D., Sahay, R., Potdar, V., Patil, D., Baradkar, S., Kumar, A. and Aggarwal, N., 2021. Isolation and characterization of SARS-CoV-2 VOC, 20H/501Y. V2, from UAE travelers, bioRxiv.
c. Yadav, P.D., Nyayanit, D.A., Sahay, R.R., Sarkale, P., Pethani, J., Patil, S., Baradkar, S., Potdar, V. and Patil, D.Y., 2021. Isolation and characterization of the new SARS-CoV-2 variant in travelers from the United Kingdom to India: VUI-202012/01 of the B. 1.1. 7 lieage. *Journal of Travel Medicine*, 28(2), p.taab009.
d. Yadav, P.D., Nyayanit, D.A., Sahay, R.R., Shete, A.M., Majumdar, T., Patil, S., Patil, D.Y., Gupta, N., Kaur, H., Aggarwal, N. and Vijay, N., 2021. Imported SARS-CoV-2 V501Y. V2 variant (B. 1.351) detected in travelers from South Africa and Tanzania to India. *Travel Medicine and Infectious Disease*.

TO:
National Lead Office FOI, DP and Records Management,
HSE National Communications Division,
Tullamore, Co Offaly.

Dear…

Request under the Freedom of Information Act 2014.

Please provide me with a full, accurate and complete list of records held by the Health Service Executive (HSE) or under the authority of the HSE which describe the isolation of the SARS-COV-2 virus (Coronavirus COVID-19), taken directly from a symptomatic patient with COVID-19, where the sample was not combined or mixed with any other source of genetic material (such as, for example, monkey kidney cells or cancer cells), thereby eliminating contamination as a possible alternative source of sampling.

Please note that the word "isolation" is used here in the normally understood meaning of that word, namely, the act of separating one thing from another. I am not referring to (and hence not requesting) documents where "isolation" means the preparation of a culture of something else, the performance of an amplification test (e.g. a PCR test which detects only mRNA or DNA), or to the sequencing of anything other than the viral isolate in question.

If any records of the HSE match the above description, please provide enough information so that I may identify and access each record with certainty. Please provide also the title, author, date, journal, weblink etc. of any document, online or otherwise, recorded on a document held by the HSE or under its authority, which describes the isolation procedure in question.

I would remind you that a full, accurate and complete disclosure is required.

Yours sincerely

Robert Pye
[redacted]
[redacted]
[redacted]
30 October 2020
Email: [redacted]

RESPONSE by HSE (23 December 2020) [C839/20]:

"Following consultation with my colleagues, both from the scientific and medical areas of HPSC [we] can confirm that we would hold no records in relation to your request."

APPENDIX B

Feidhmeannacht na Seirbhíse Sláinte
Health Service Executive

hpsc

23rd December 2020

C839/20

Mr Robert Pye

Re: FOI request C839/20

Dear Mr Pye

I refer to your request which was received by this office on 22nd December 2020 which you have made under the Freedom of Information Act 2014 for records held by this FOI body. Your request sought the provision of the following information with regards to

"A full, accurate and complete list of records held by the Health Service Executive (HSE) or under the authority of the HSE which describe the isolation of the SARS-COV-2 virus (Coronavirus COVID-19), taken directly from a symptomatic patient with COVID-19, where the sample was not combined or mixed with any other source of genetic material (such as, for example, monkey kidney cells or cancer cells), thereby eliminating contamination as a possible alternative source of sampling.

Please note that the word "isolation" is used here in the normally understood meaning of that word, namely, the act of separating one thing from another. I am not referring to (and hence not requesting) documents where "isolation" means the preparation of a culture of something else, the performance of an amplification test (e.g. a PCR test which detects only mRNA or DNA), or to the sequencing of anything other than the viral isolate in question.

If any records of the HSE match the above description, please provide enough information so that I may identify and access each record with certainty. Please provide also the title, author, date, journal, weblink etc. of any document, online or otherwise, recorded on a document held by the HSE or under its authority, which describes the isolation procedure in question.

I would remind you that a full, accurate and complete disclosure is required".

Following consultation with my colleagues, both from the scientific and medical areas of HPSC can confirm that we would hold no records in relation to your request. These are the reasonable steps I have taken to ascertain the whereabouts or existence of such records and unfortunately I must inform you that having undertaken these searches we were unable to locate the records in question. I am satisfied that all reasonable steps have taken to locate the records you have requested and must refuse therefore your request under Section 15.1(a) of the FOI Act 2014.

Rights of appeal

In the event that you are unhappy with this decision you may appeal this it. In the event that you need to make such an appeal, you can do so by writing to the HSE National Lead Office, FOI, DP and Record Management, Scott Building, Midland Regional Hospital Campus, Arden Road, Tullamore, Co. Offaly. Your correspondence should include a fee of €30 for processing the appeal. An internal review fee of €10 applies to medical card holders. Payment should be made by way of bank draft, money order, postal order or personal cheque made payable to the Health Service Executive. If you wish to make payment by electronic means please contact emma.kelly@hse.ie . You should make your appeal within 4 weeks from the date of this notification, where a day is defined as a working day excluding, the weekend and public holidays. However, the making of a late appeal may be permitted in appropriate circumstances. The appeal will involve a complete reconsideration of the matter by a more senior member of the staff of this body.

Should you have any questions or concerns regarding the above, please contact me by email on sinead.roche@hse.ie.

Yours sincerely,

Sinead Roche

FOI Decision Maker

Oifig Dli UCD

Caisleán an Ruabhoic,
An Coláiste Ollscoile, Baile Átha Cliath,
Belfield, Baile Átha Cliath 4, Eire

corporate.legal@ucd.ie
www.ucd.ie/corpsec

UCD Legal Office

Roebuck Castle.
University College Dublin,
Belfield, Dublin 4, Ireland

T +353 1 716 8708

Mr James McCumiskey
By email: jl_mccumiskey@yahoo.ie

22 June 2020

Reference: FOI12_1_544 Internal Review

Dear Mr McCumiskey,

I refer to your application for an internal review under the Freedom of Information Act 2014 of a decision by Ms Debbie Scanlan, dated 22 May 2020, concerning item 1 of your request for access to records of the National Virus Reference Laboratory (NVRL), as follows: *"1) I am looking for a scientific paper, which demonstrates how the Novel Coronavirus was purified? Surely, if the NVRL is able to detect the Novel Coronavirus, it should also be able to demonstrate how it is purified?"*

In the original decision, Ms Scanlan refused part 1 of your request on grounds that the University do not hold records to answer your request (Section 15 (1) (a)).

I have now conducted an internal review in accordance with Section 21 of the Act. I wish to inform you that I affirm the original decision.

The University's position is that matters of academic debate cannot be conducted under FOI and we would not regard academic research material as administrative records of an FOI body that would make them available for release under the legislation. The NVRL have advised that they do not culture live SARS-CoV-2 or purify SARS CoV 2 antigens. They detect SARS-CoV-2 RNA in diagnostic samples, as per the PCR assay that was shared with you previously. As such, there are no relevant records held and no further searches that may be taken for records that would provide an answer to your query. Section 15 (1) (a) of the FOI applies.

The University is committed to its obligations under the Act to provide requesters with access to records held by it and with reasons for its decisions that affect them. In this case, we regret that we cannot assist you further.

Under the Act, the University is required to advise you of your right, following receipt of your internal review decision, to make a further review application by writing to the Information Commissioner, 18 Lower Leeson Street, Dublin 2.

Yours sincerely,

Mr Julian Bostridge
Director of Legal Services

Isle of Man Government
Reiltys Ellan Vannin

Department of Health and Social Care

Rheynn Slaynt as Kiarail y Theay

Mr Steven Gardner
39 Princes Street
Douglas
Isle of Man
IM1 1BB

Interim Chief Executive: Kathryn Magson
Freedom of Information Team
Crookall House
Demesne Road
Douglas
Isle of Man
IM1 3QA

Tel: (01624) 642621
Email: dhsc@foi.gov.im
Website: www.gov.im/dhsc

Our ref: 1646813

18th February 2021

Dear Mr Gardner

We write further to your request which was received on the 26th January 2021 and states:

Question 1:
Has Covid 19/21 been isolated?

Question 2:
Has covid 19/21 been purified?

Question 3:
Has there been a risk assessment on masks?

Question 4:
Have all places of business who have mandatory masks done a risk assessment or should they do a risk assessment, in regards to masks? For their employees and customers.

Question 5:
Is the sequence in the PCR test SarsCov2?

Question 6:
What amplifications has the PCR test been run at?

Question 7:
Can you provide the season flu death numbers for 2019 & 2020?

Clarification sought:
Regarding questions 1 & 2 when you say 'Has Covid 19/21 been isolated' do you mean has SARS-CoV-2 been isolated? If you don't please can you clarify what you are referring to?

Clarification received:
Yes, SarsCov2 has it been isolated and purified.

Our response:

Clarification sought:
Regarding questions 1 & 2 when you say 'Has Covid 19/21 been isolated' do you mean has SARS-CoV-2 been isolated? If you don't please can you clarify what you are referring to?

Clarification received:
Has the SarsCov2 been isolated and purified. To be proven scientifically and proven the virus causes disease.

Question 1:
Has Covid 19/21 been isolated?
Regarding SARS-CoV-2 the virus is not isolated.

Question 2:
Has covid 19/21 been purified?
Regarding SARS-CoV-2 it is not purified.

Question 3:
Has there been a risk assessment on masks?
The Department has and does risk assessments on masks.

Question 4:
Have all places of business who have mandatory masks done a risk assessment or should they do a risk assessment, in regards to masks? For their employees and customers.
While our aim is to provide information whenever possible, in this instance the Department of Health and Social Care ("the Department") is unable to provide the information that you have requested. This is in line with Section 11(3)a of the Act, as a practical refusal reason applies; namely we do not hold or cannot, after taking reasonable steps to do so, find the information that you have requested.

Places of business are responsible for undertaking their own risk assessments and setting their own policies for wearing masks.

To provide further advice and assistance guidance on face coverings, including 'face coverings at work' is available within the public domain at:
https://covid19.gov.im/general-information/guidance-on-face-coverings/

Question 5:
Is the sequence in the PCR test SarsCov2?
Yes, the sequence in the PCR test is SARsCov2

Question 6:
What amplifications has the PCR test been run at?
The amplification is 45 cycles.

Question 7:
Can you provide the season flu death numbers for 2019 & 2020?
While our aim is to provide information whenever possible, in this instance the Department of Health and Social Care ("the Department") is unable to provide the information that you have requested. This is in line with Section 11(3)a of the Act, as a practical refusal reason applies; namely we do not hold or cannot, after taking reasonable steps to do so, find the information that you have requested.

However you may wish to re-submit your request to Public Health within the Cabinet Office who may be able to help you. The information you have requested is held by Public Health.

Please quote the reference number 1646813 in any future communications.

Your right to request a review

If you are unhappy with this response to your freedom of information request, you may ask us to carry out an internal review of the response, by completing a complaint form and submitting it electronically or by delivery/post.

An electronic version of our complaint form can be found by going to our website at https://services.gov.im/freedom-of-information/Review . If you would like a paper version of our complaint form to be sent to you by post, please contact me and I will be happy to arrange for this. Your review request should explain why you are dissatisfied with this response, and should be made as soon as practicable. We will respond as soon as the review has been concluded.

If you are not satisfied with the result of the review, you then have the right to appeal to the Information Commissioner for a decision on;

1. Whether we have responded to your request for information in accordance with Part 2 of the Freedom of Information Act 2015; or
2. Whether we are justified in refusing to give you the information requested.

In response to an application for review, the Information Commissioner may, at any time, attempt to resolve a matter by negotiation, conciliation, mediation or another form of alternative dispute resolution and will have regard to any outcome of this in making any subsequent decision.

More detailed information on your right to a review can be found on the Information Commissioner's website at www.inforights.im.

Should you have any queries concerning this letter, please do not hesitate to contact me.

Further information about freedom of information requests can be found at www.gov.im/foi.

I will now close your request as of this date.

Yours sincerely

Debbie Hay
FOI Coordinator

Gazzo, lì 07/10/2021

Istanza per l'accesso agli atti amministrativi ai sensi dell'art. 5, comma 2, del D.Lgs. 33/2013, come modificato dal D.Lgs. 97/2016

BUFFONATE DI STATO

Al Segretariato generale

del Ministero della Salute

e per conoscenza: - protocollo.centrale@pec.iss.it

- urp@inmi.it
- protocollo.aopd@percveneto.it
- direzione.medicinamolecolare@unipd.it

Il sottoscritto [redacted]

CHIEDE

ai sensi e per gli effetti dell'art. 5, comma 2 e ss. del D.Lgs. n. 33/2013, come modificato dal D.Lgs. n. 97/2016:

– di ottenere copia semplice in formato digitale .pdf oppure .docx dei seguenti documenti:

documentazione riguardante qualsiasi studio o report relativo all'isolamento e/o purificazione del virus Sars-Cov2 e qualunque variante (ad esempio tramite filtrazione, ultracentrifugazione o cromatografia), ottenuti a partire da un campione prelevato da un malato, non combinato con altre sorgenti di materiale genetico.

In attesa di un vostro sollecito riscontro porgo distinti saluti

Firma del richiedente [redacted]

0049278-29/10/2021-DGPRE-I

[redacted]

Ministero della Salute

DIREZIONE GENERALE DELLA PREVENZIONE SANITARIA
Ufficio 1 – Affari Generali e Segreteria Tecnico Organizzativa
Viale Giorgio Ribotta, 5 – 00144 Roma

e p.c.

ALL'UFFICIO DI GABINETTO
gab@postacert.sanita.it

Registro – classif:DGPREV/I/ I.8.e.c.3

AL SEGRETARIATO GENERALE
SEGGEN

BUFFONATE DI STATO

AL RESPONSABILE DELLA TRASPARENZA

SEDE

Oggetto: Istanza di accesso civico generalizzato [redacted]

Si fa riferimento all'istanza di cui all'oggetto, con la quale la S.V. chiede la documentazione riguardante "qualsiasi studio o report relativo all'isolamento e/o purificazione del virus Sars-Cov2 e qualunque variante (ad esempio tramite filtrazione, ultracentrifugazione o cromatografia), ottenuti a partire da un campione prelevato da un malato, non combinato con altre sorgenti di materiale genetico".

In merito, si rappresenta che il Decreto Legislativo 14 marzo 2013, n. 33, all'art. 5 (Accesso civico a dati e documenti), prevede al comma 2 che "chiunque ha diritto di accedere ai dati e ai documenti detenuti dalle pubbliche amministrazioni, ulteriori rispetto a quelli oggetto di pubblicazione ai sensi del presente decreto, nel rispetto dei limiti relativi alla tutela di interessi giuridicamente rilevanti secondo quanto previsto dall'articolo 5-bis"ma, come chiarito dall'ANAC [1], "resta escluso che – per rispondere a tale richiesta – l'amministrazione sia tenuta a formare o raccogliere o altrimenti procurarsi informazioni che non siano già in suo possesso, pertanto, l'amministrazione non ha l'obbligo di rielaborare i dati ai fini dell'accesso generalizzato, ma solo consentire l'accesso ai documenti nei quali siano contenute le informazioni già detenute e gestite dall'amministrazione stessa."

Ciò posto, in un'ottica di leale collaborazione e trasparenza, Le segnaliamo la pagina dell'Istituto Superiore di Sanità (ISS) sulle varianti[2] ed, in particolare, l'ultima *survey* sull'argomento[3], nonché, sul sito *web* di questo Ministero, la pagina con le domande frequenti (*Frequently Asked Questions* -FAQ) e le risposte sulle varianti[4].

Distinti saluti

Il Direttore generale
F.to Dott. Giovanni Rezza*

* *Firma autografa sostituita a mezzo stampa ai sensi dell'art.3, comma 2, del D.lgs. n.39/1993*

[1] ANAC, *Linee guida* 1309/2016 https://www.anticorruzione.it/-/determinazione-n.-1309-del-28/12/2016-rif.-1?inheritRedirect=true&redirect=%2Fconsulta-i-documenti%3Fstart%3D2%26q%3D%2522Determinazione%2520n.%25201309%2522%26sort%3Dddm__Dataclu0_String_sortable-
[2] https://www.iss.it/cov19-cosa-fa-iss-varianti
[3] https://www.iss.it/documents/20126/0/Relazione+tecnica+quick+survey+varianti+settembre+2021+versione+finale.pdf/00430078-505f-082f-20e6-6ad2a6312c68?t=1633703643180
[4] https://www.salute.gov.it/portale/nuovocoronavirus/dettaglioFaqNuovoCoronavirus.jsp?lingua=italiano&id=250

Subject: Request for generalised public access

We refer to the above mentioned request, by which you require the documentation concerning "any study or report about the isolation and/or purification of the Sars-Cov-2 virus and any variant (i.e. by filtration, ultracentrifugation or chromatography), obtained from a sample taken from a deseased human, not combined with other sources of genetic material".

In this regard, we point out that the Legislative Decree no. 33 of March 14, 2013 art. 5 (Public access to data and documents), in subsection 2 states that "anyone has the right to access the data and documents held by public administrations, in addition to those subject to publication in accordante with this decree, in compliance with the limits related to the protection of legally significant interests as provided in article 5-bis" but, as clarified by ANAC (1), "it is excluded that - to respond to such a request - the administration is required to produce or collect or otherwise obtain information that is not already in its possession, therefore, the administration is not obliged to work on the data for the purposes of generalized access, but only allow access to documents containing the information already held and managed by the administration itself. "

That said, in a perspective of fair cooperation and transparency, we recommend you the page of the Institute of Health (ISS) on variants (2) and, in particular, the latest survey on the matter (3), as well as, on the website of this Ministry, the page with Frequently Asked Questions (FAQs) and answers about variants (4).

Best Regards

https://www.anticorruzione.it/-/determinazione-n.-1309-del-28/12/2016-rif.-1?inheritRedirect=true&redirect=%2Fconsulta-i-documenti%3Fstart%3D2%26q%3D%2522Determinazione%2520n.%25201309%2522%26sort%3Dddm__Dataclu0_String_sortable-

https://www.iss.it/cov19-cosa-fa-iss-varianti

https://www.iss.it/documents/20126/0/Relazione+tecnica+quick+survey+varianti+settembre+2021+versione+finale.pdf/00430078-505f-082f-20e6-6ad2a6312c68?t=1633703643180

https://www.salute.gov.it/portale/nuovocoronavirus/dettaglioFaqNuovoCoronavirus.jsp?lingua=italiano&id=250

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

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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.

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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****".*

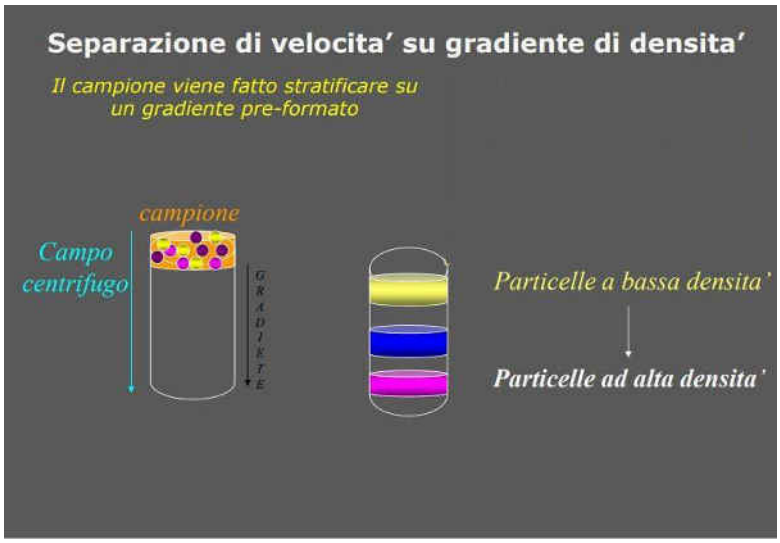
Ci ricorda anche che l'evidenza della presenza del virus è acquisita rilevando:

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.

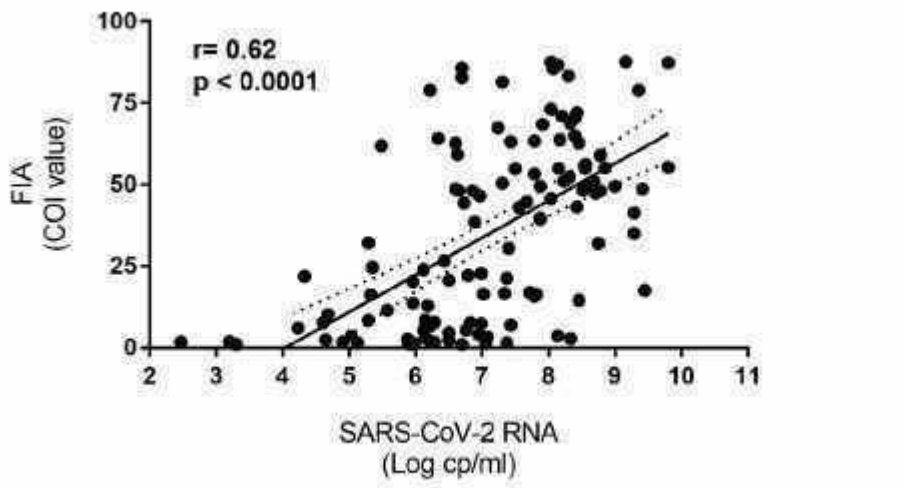


Figure 2. Correlation between SARS-CoV-2 RNA copies number and COI obtained on confirmed SARS-CoV-2 positive samples with available information for both parameters ($n = 125$, as in blue box

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.

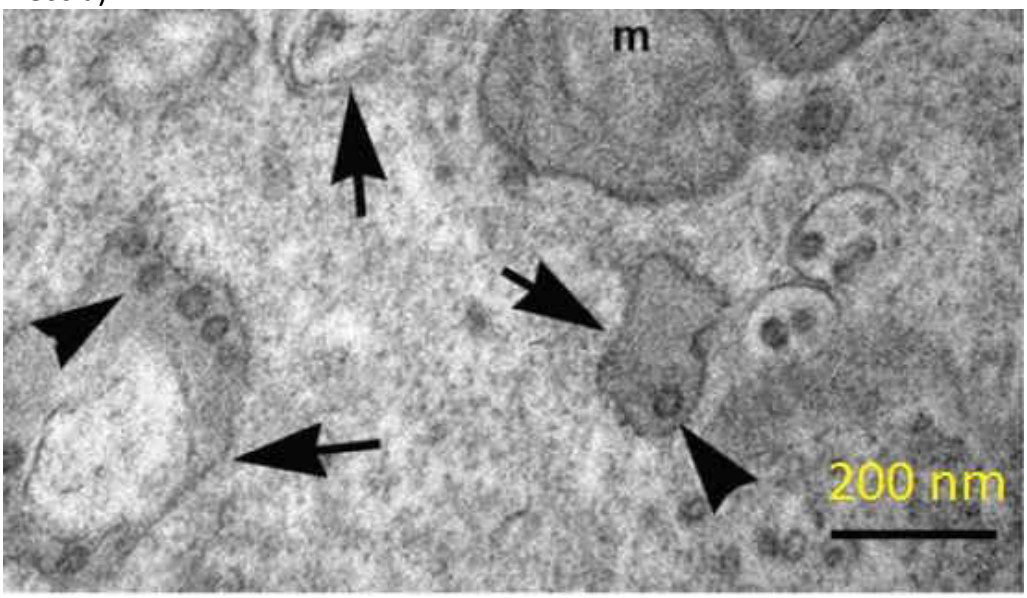
Gli autori scrivono: *"il diametro dei virus variava da 80 a 102 nm (misura media 93,61)"*.

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)



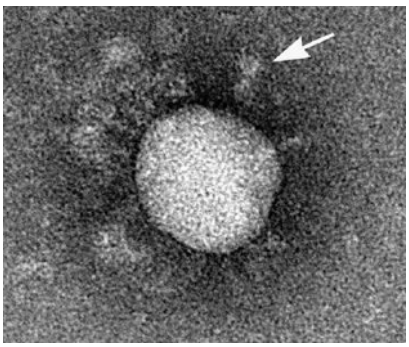
j) Nella fig 6 C: 100 nm

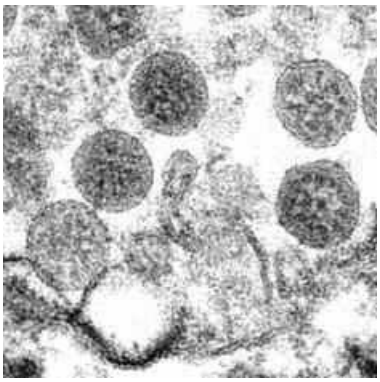
Val la pena ricordare che una particella con un raggio doppio rispetto ad un'altra ha un volume maggiore di 8 volte!

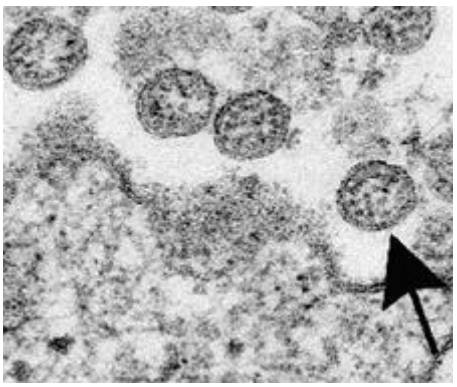
La numerosità di particelle con dimensioni ben inferiori a quelle minime attribuite ai Coronavirus e pure a quelle indicate dagli Autori, esclude si sia trattato di sviste od errori. Questo riscontro riporta in primo piano la discussione

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.

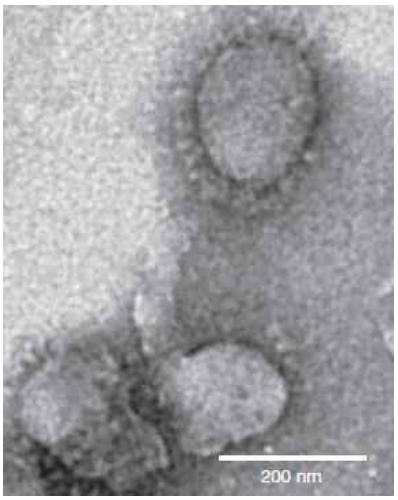
Per inciso, vi è notevole differenza anche nell'aspetto dei virioni, così come fotografati da Nardacci et al. e quelli fotografati da Goldsmith CS et al., dei CDC [48] e Hartcourt J et al, dei CDC [49], ad esempio. In questi ultimi **nessuna spike** – caratteristica da cui il coronavirus deriva il nome - 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

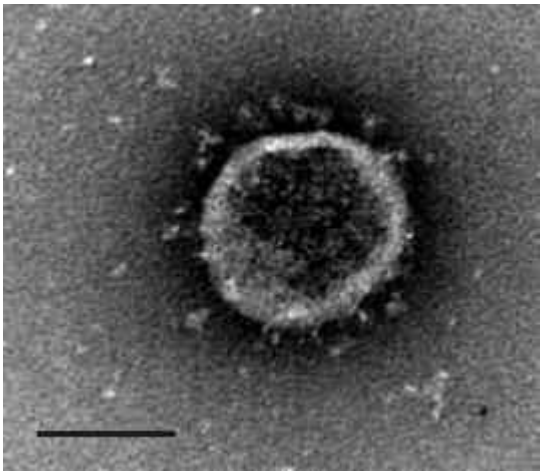
 (Hartcourt J et al) no spikes

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

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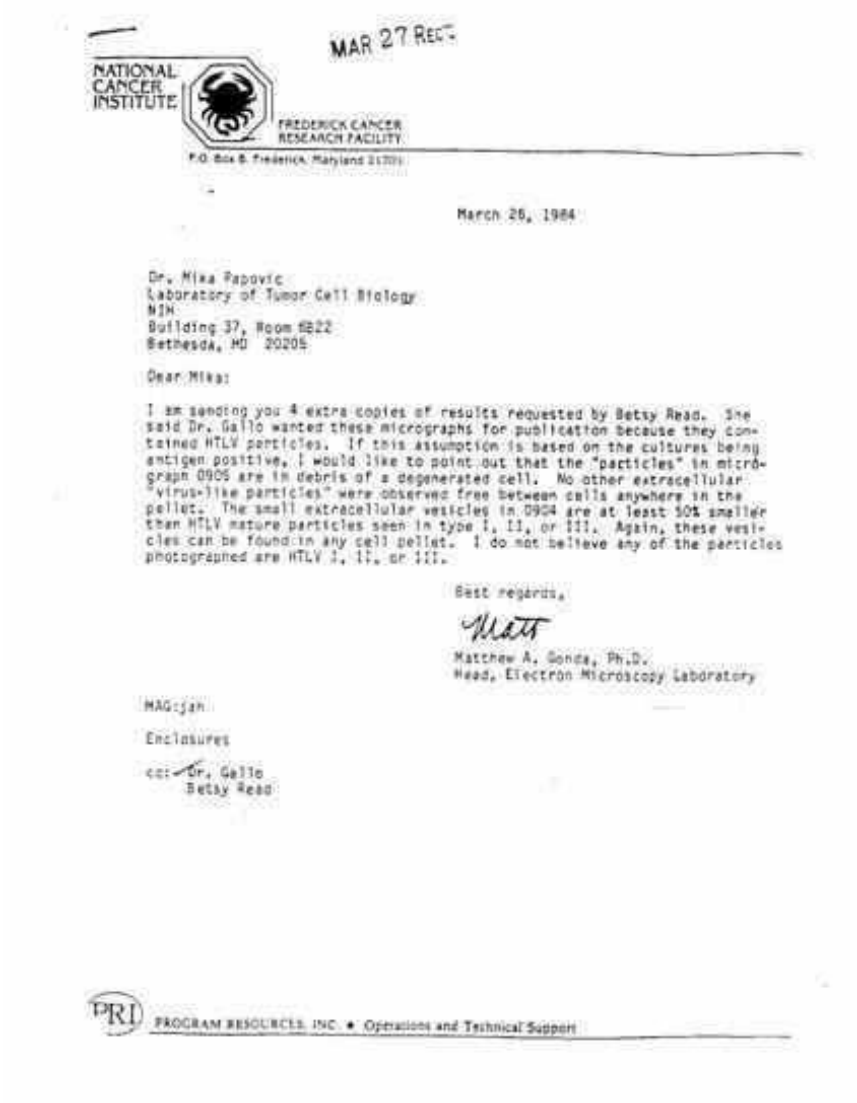


MAR 27 REC'D

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 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.
Head, Electron Microscopy Laboratory

MAG:jan

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

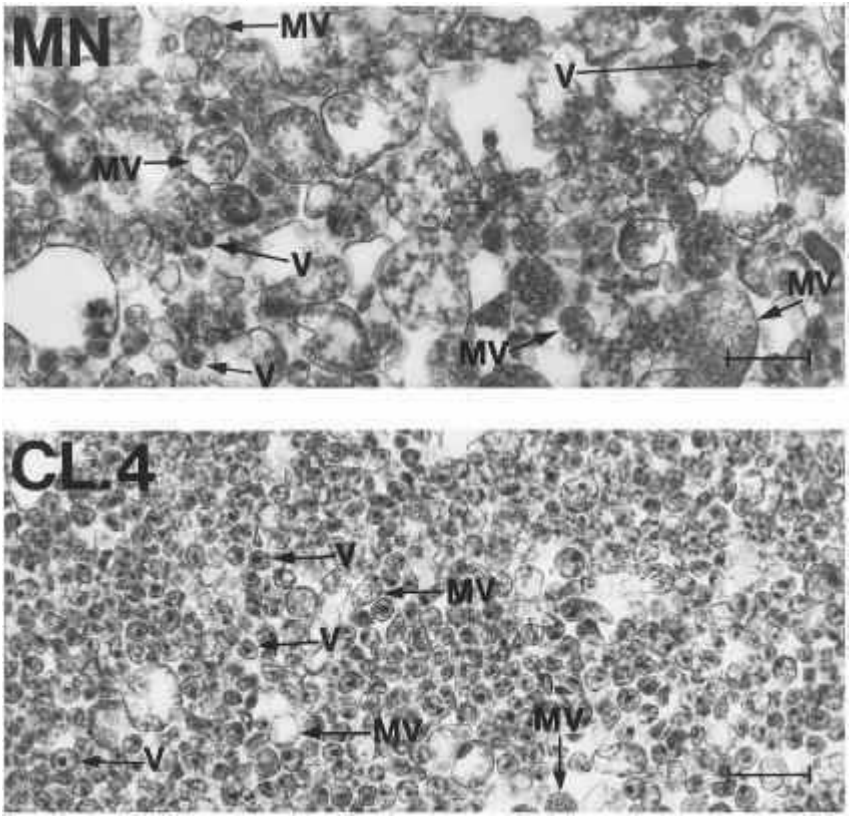
[5] Popovic M, Gallo R et al Science 1984;224:497-500

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)



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[22] Prof Ariberto Fassati. MD PhD della Division of Infection & Immunity, School of Medical Sciences, University College London
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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."**

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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. —

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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****".*

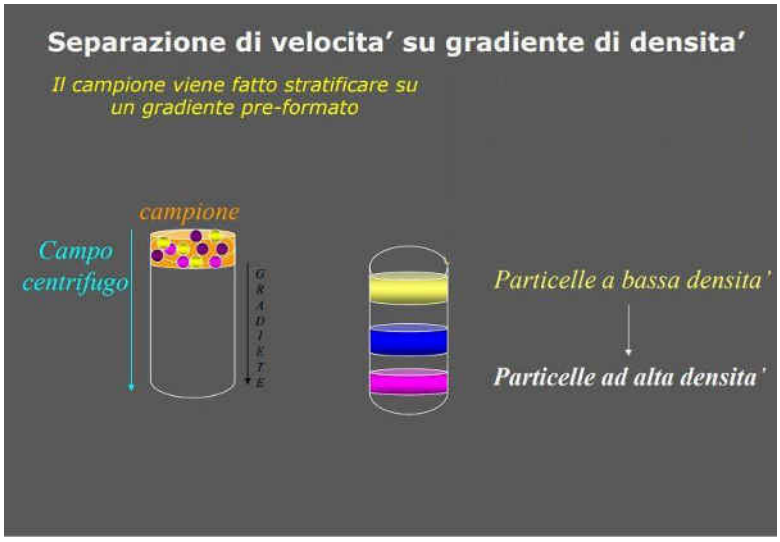
You also remind us that the evidence of the presence of the virus is acquired noting:

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:

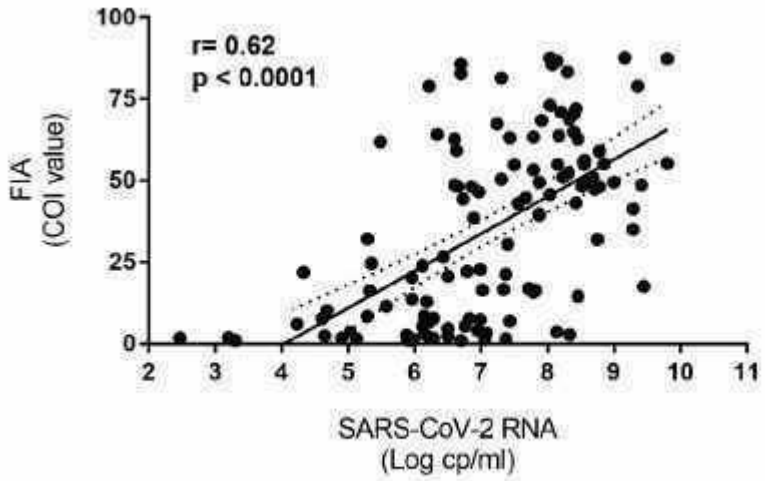


Figure 2. Correlation between SARS-CoV-2 RNA copies number and COI obtained on confirmed SARS-CoV-2 positive samples with available information for both parameters ($n = 125$, as in blue box

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.

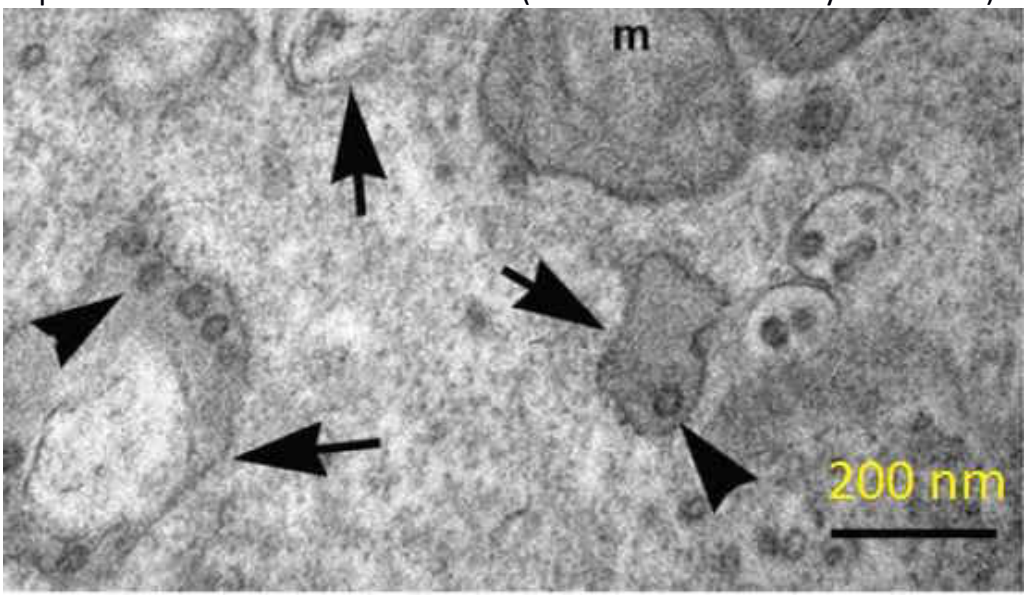
The authors write: *"the diameter of the viruses changed from 80 to 102 nm (average size 93,61)"*.

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)

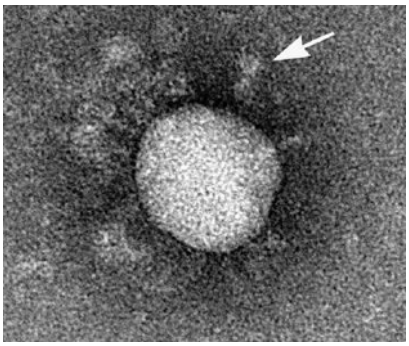


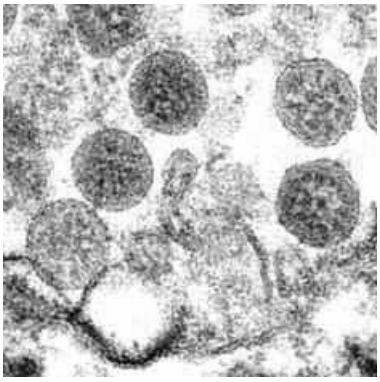
j) In pict. 6 C: 100 nm

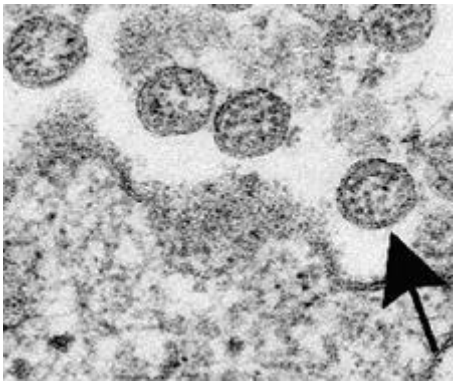
It is important to recall that a particle with a radius twice as large as another one has a volume 8 times bigger!

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.

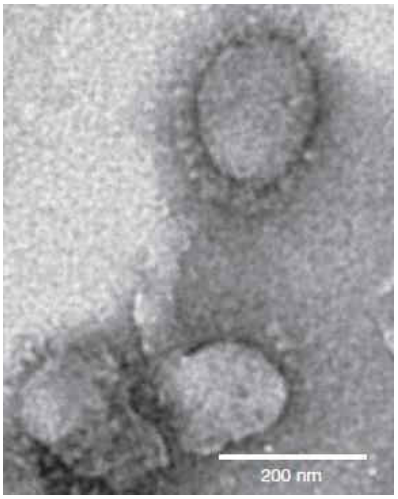
Incidentally, there is also a huge difference in the virions' aspect, as they were photographed by Nardacci et al. and the ones photographed by Goldsmith CS et al., by CDC [48] and Hartcourt J et al, by CDC [49], for example. In these last ones **no spike – characteristic from which coronavirus derives 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

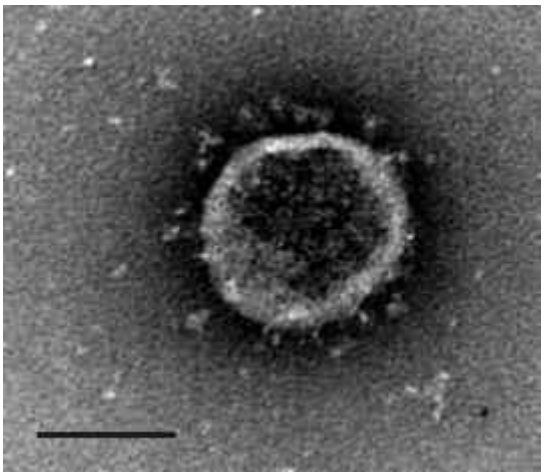

(Hartcourt J et al) no spikes

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

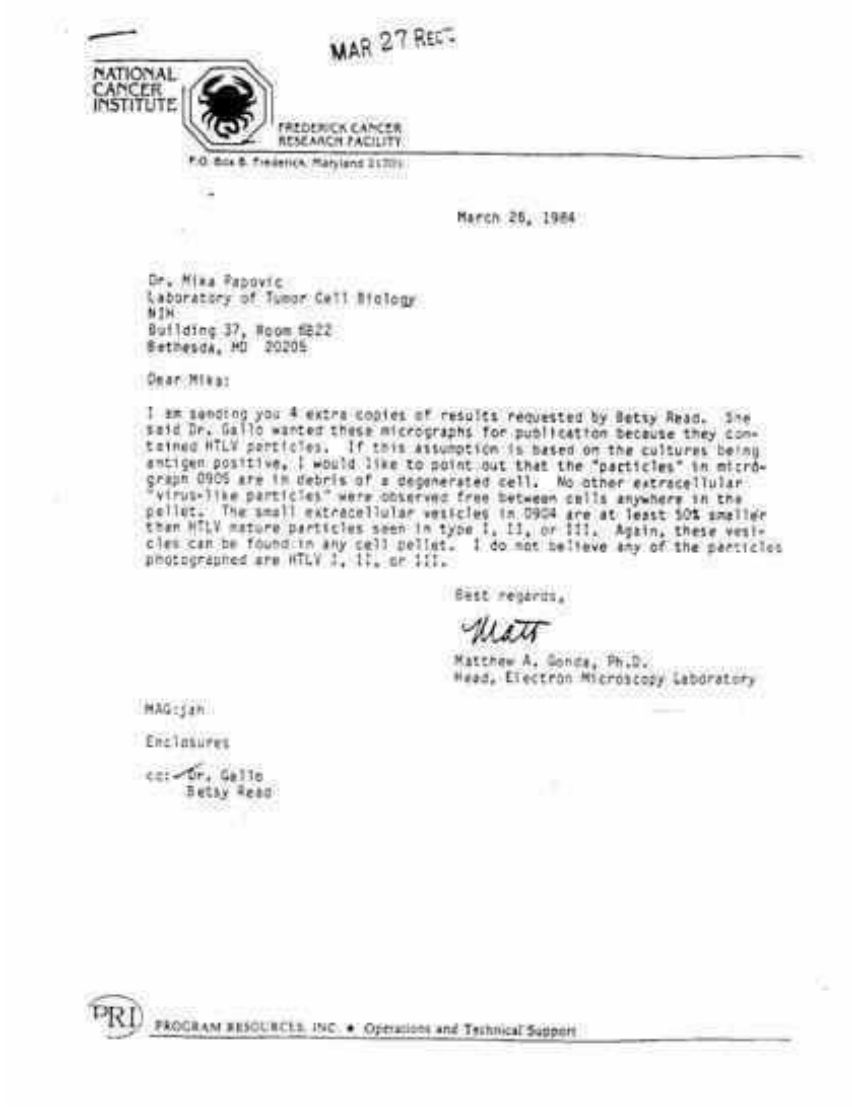
Stefano Scoglio. The final evidence (La prova definitiva), 8th June 2021

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 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.
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

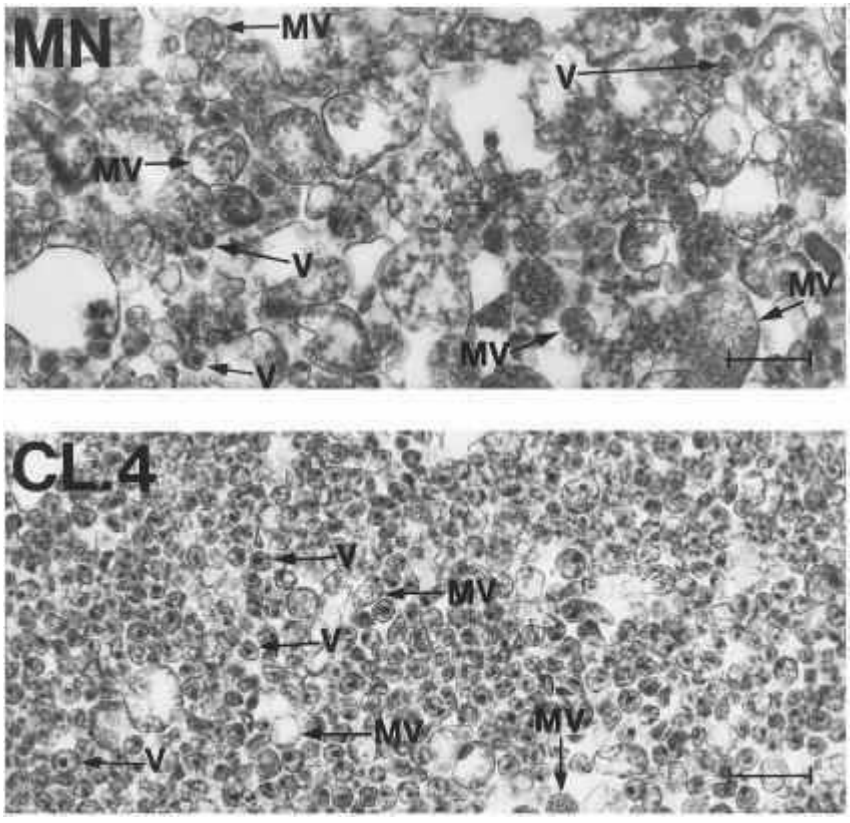
5 Popovic M, Gallo R et al., Science 1984;224:497-500

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
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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."**

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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. —

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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).

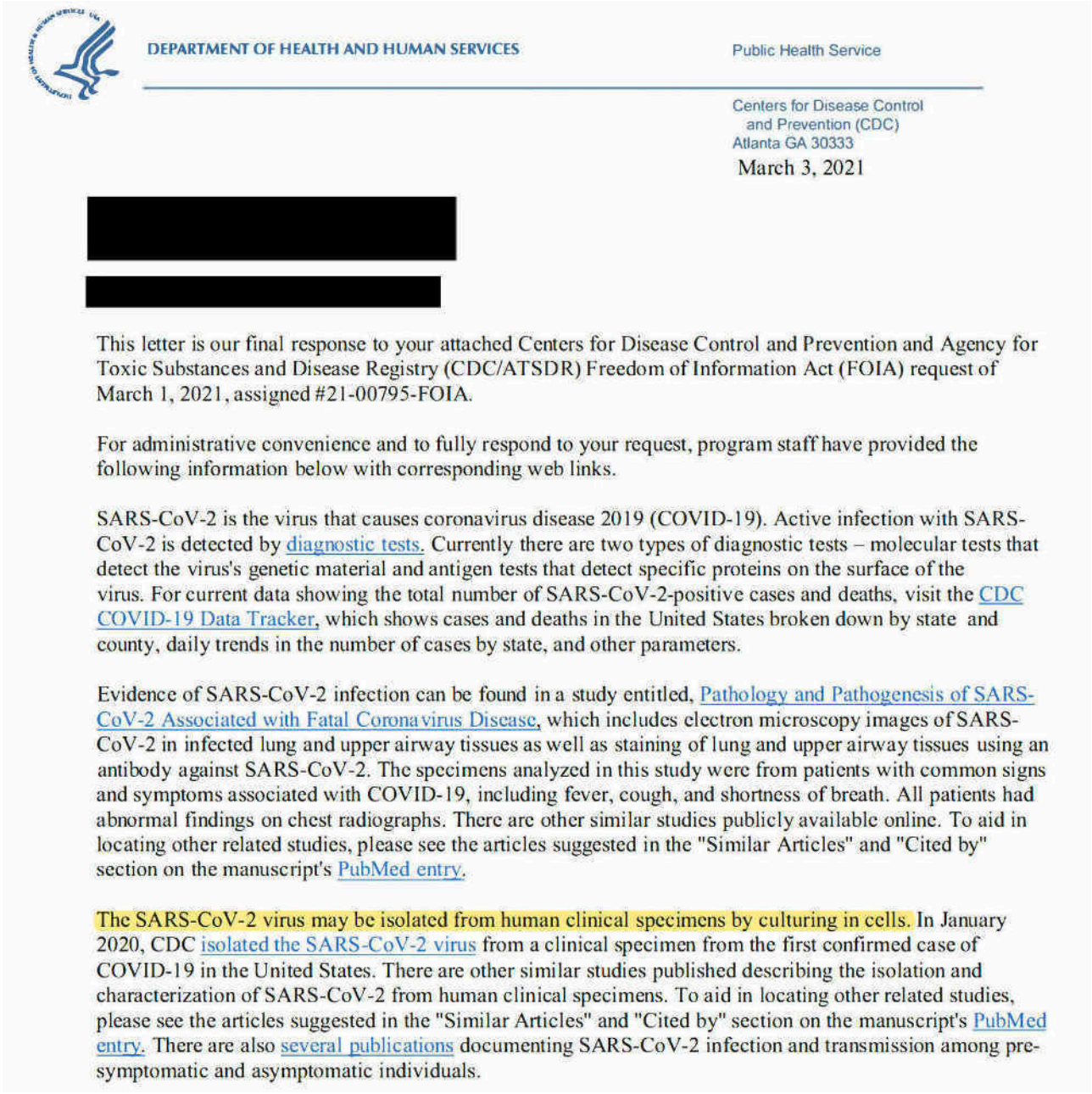
costituisce il 100% del nuovo materiale isolato che si ottiene. Può darsi che non si possa raggiungere il 100% per la presenza di qualche impurità, ma comunque si parlerebbe di un isolato al +/- 95%. Questo non sarebbe ideale, perché se io devo essere certo che un certo batterio o "virus" sia patogeno, 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

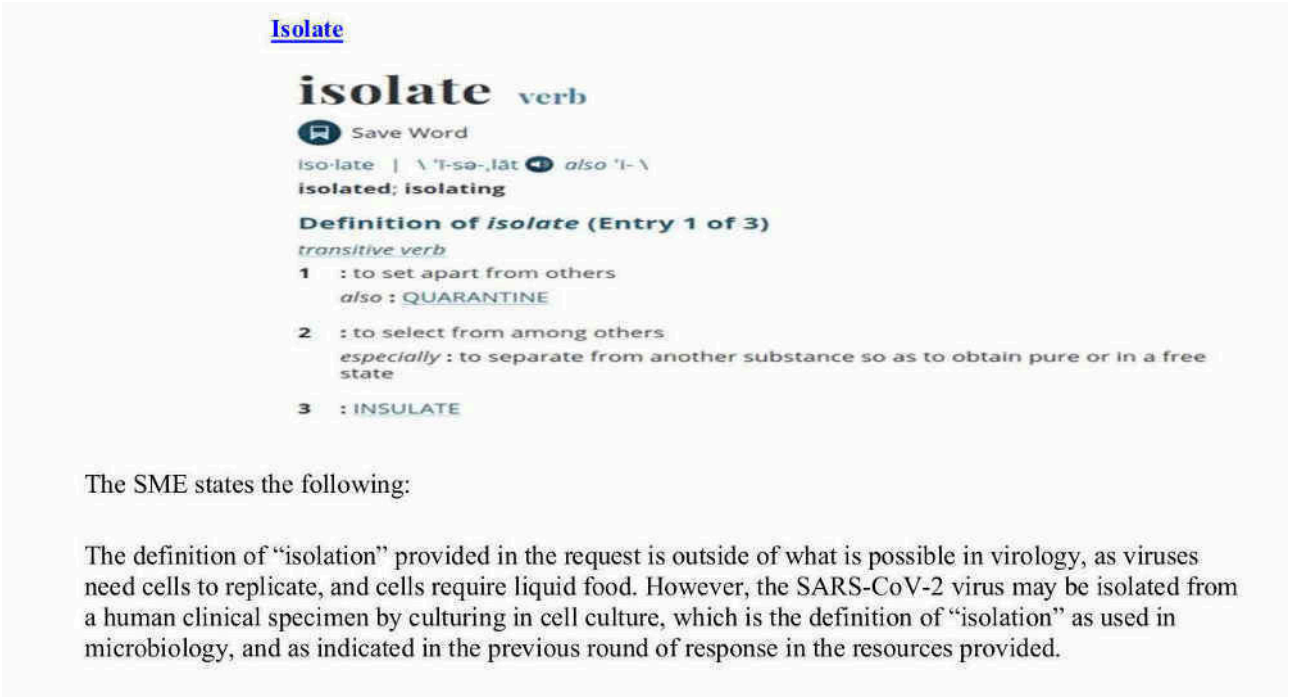
Quindi, la richiesta è specifica, e si chiede se il virus è stato isolato secondo la definizione comune di "isolamento", come riportata nel vocabolario:

"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 ˈī- \

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:

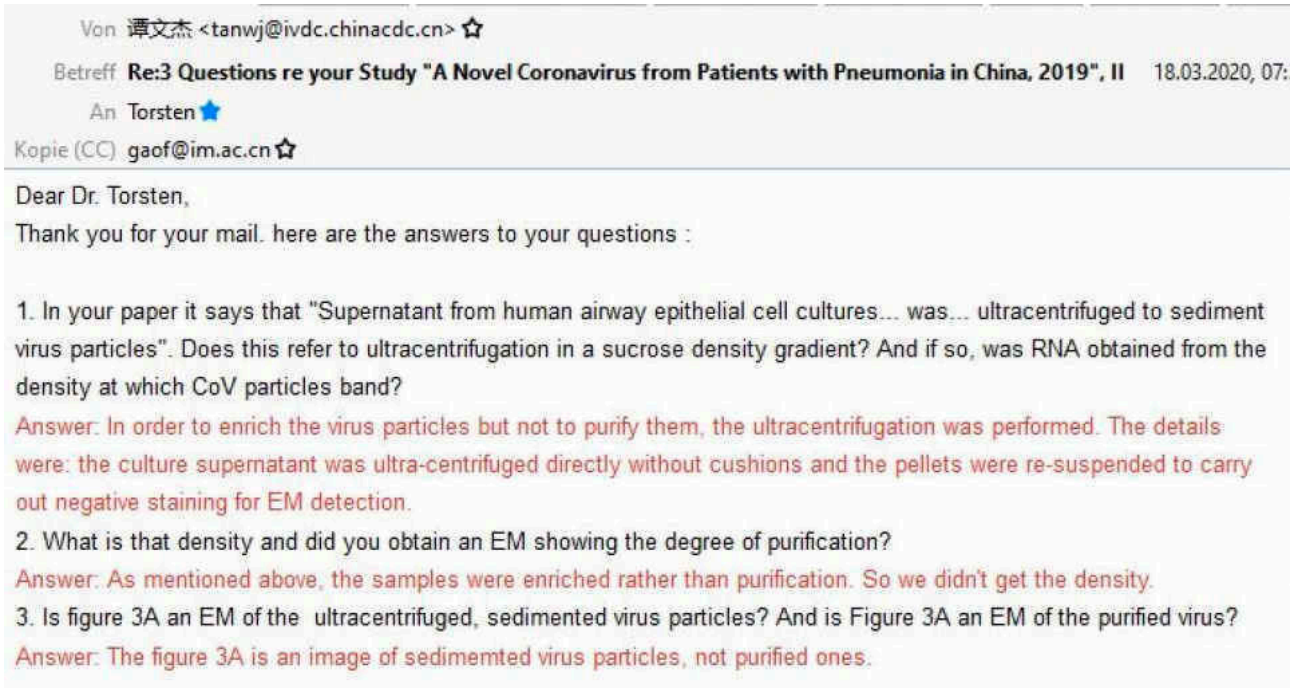
The definition of "isolation" provided in the request is outside of what is possible in virology, as viruses need cells to replicate, and cells require liquid food. However, the SARS-CoV-2 virus may be isolated from a human clinical specimen by culturing in cell culture, which is the definition of "isolation"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

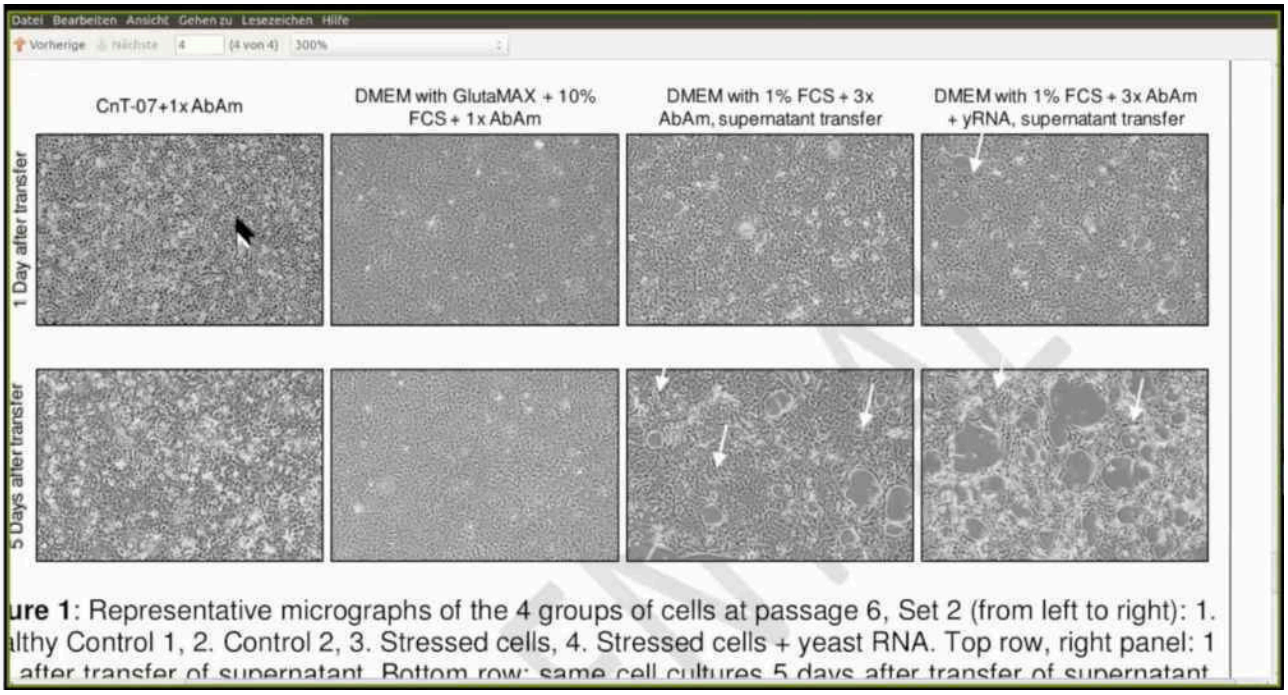
[5] Li P. et al., Progress in Exosome Isolation Techniques, Theranostics. 2017; 7(3): 789–804.

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".

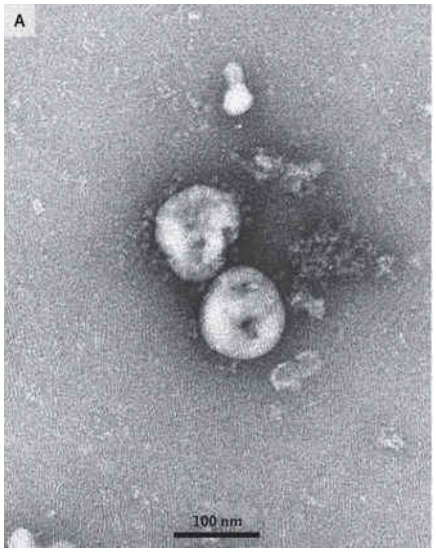
Le diapositive del dr. Lanka mostrano nella banda superiore 4 stadi di trattamento delle cellule Vero al giorno 1, e nella banda sottostante gli stessi 4 stadi al giorno 5. I 4 stadi della procedura sono gli stessi utilizzati in virologia, e simili a quelli descritti nell'articolo del CDC riportato sopra, 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:

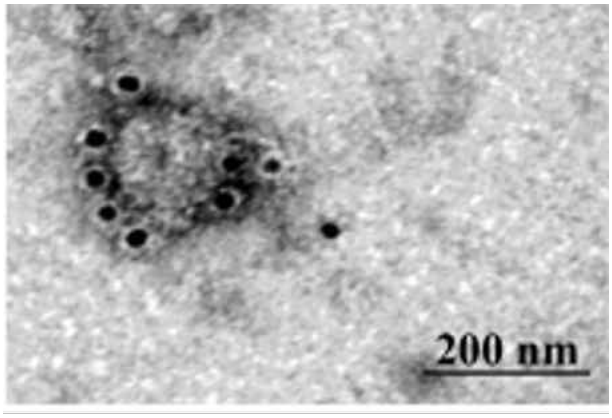


Foto EM di esosoma

[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:

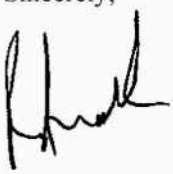
The requester specifies that the requester would like documents related to isolation, defined by the requester as "separation of SARS-COV-2 from everything else also known as purification"; viruses need cells to replicate, and cells require liquid food, so this specific component of the request is outside 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];

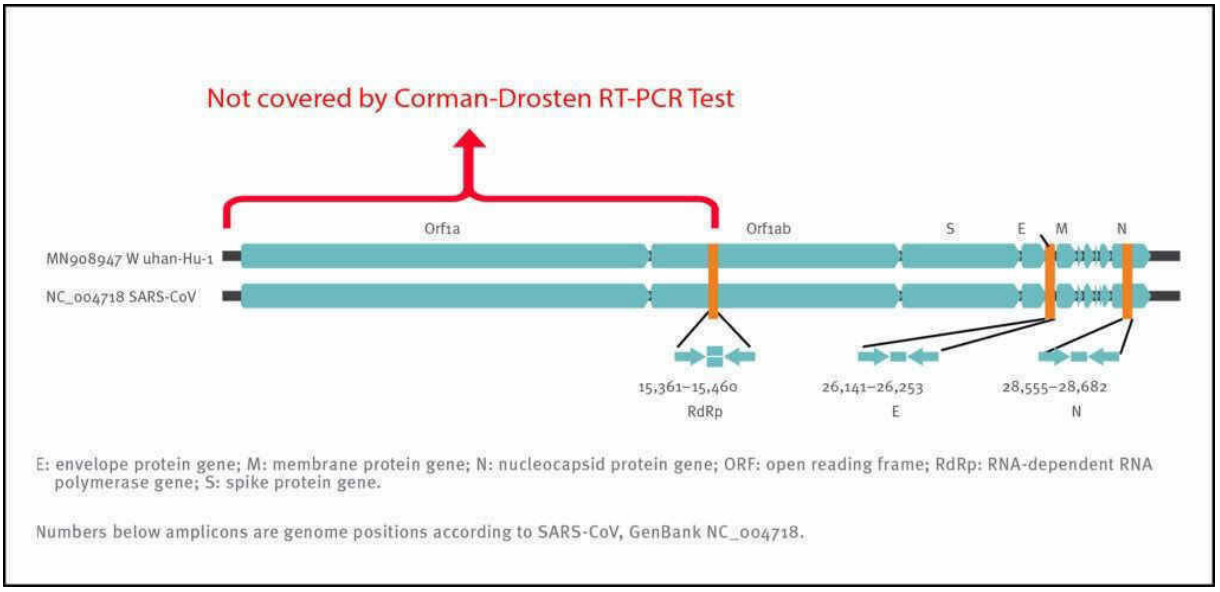
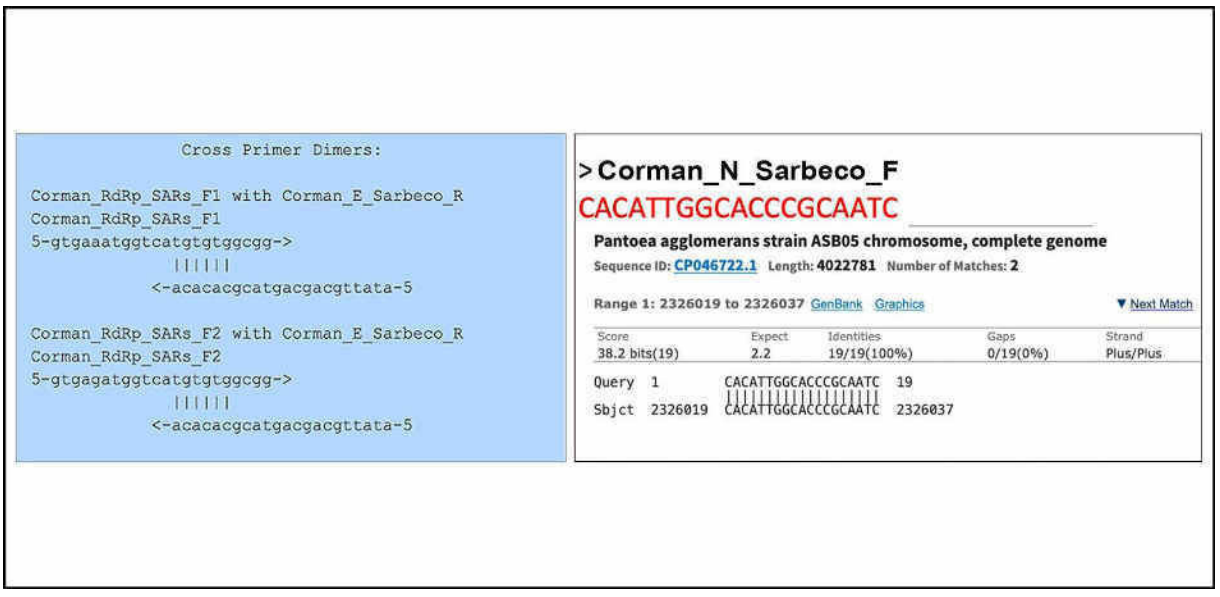


Figure 3: A test with Thermofischer's primer dimer web tool reveals that the RdRp forward primer has a 6bp 3`prime homology with Sarbeco E Reverse (left box). Another test reveals that there is a perfect match for one of the N-primers to a clinical pathogen (Pantoea) found in immuno-compromised patients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

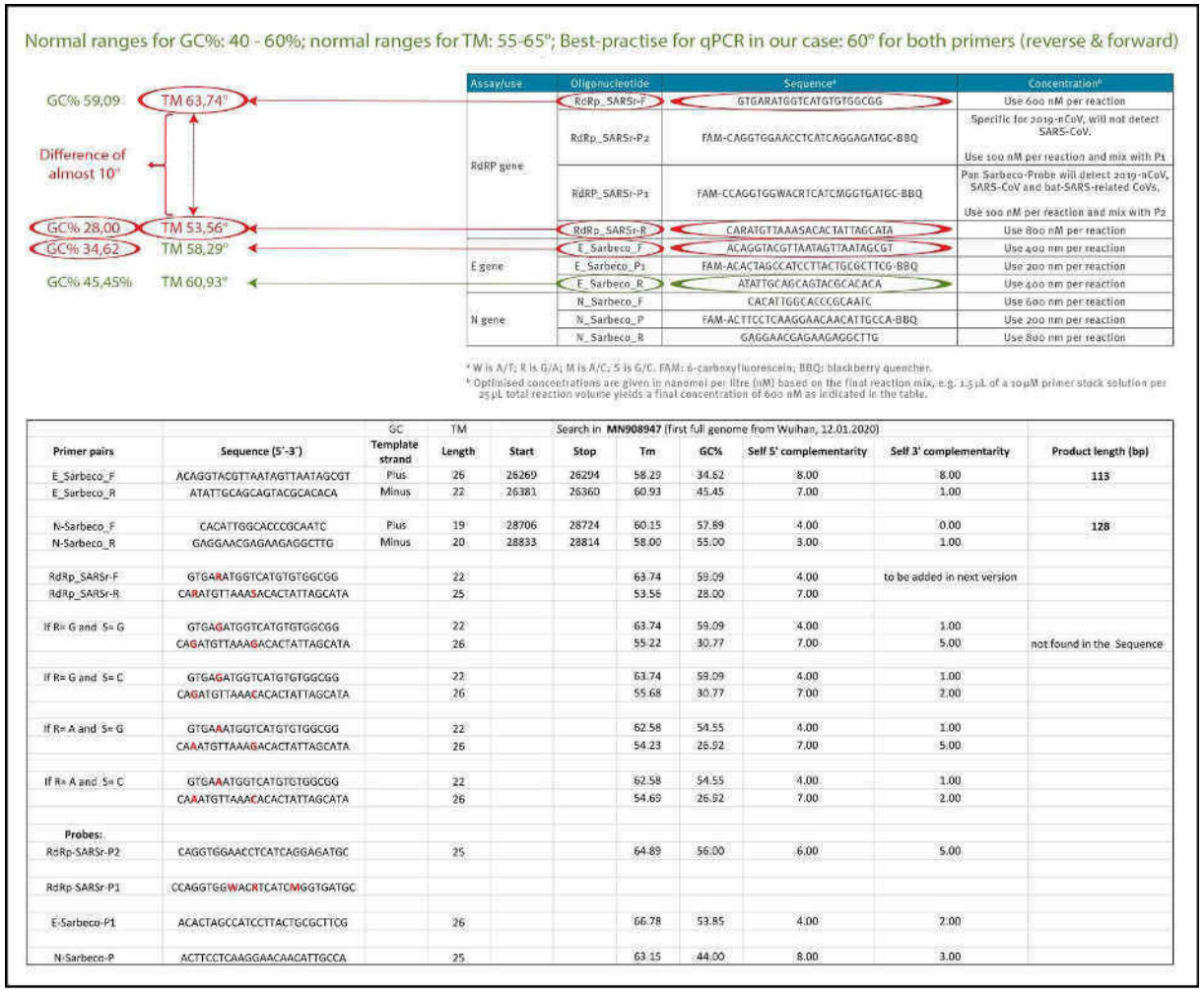
The annealing temperature (Tm) is a crucial factor for the determination of the specificity/accuracy of the qPCR procedure and essential for evaluating the accuracy of qPCR-protocols. Best-practice recommendation: Both primers (forward and reverse) should have an almost similar value, preferably the 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 10µM primer stock solution per 25 µL total reaction volume yields a final concentration of 600 nM as indicated in the table.

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

3. The number of amplification cycles

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

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

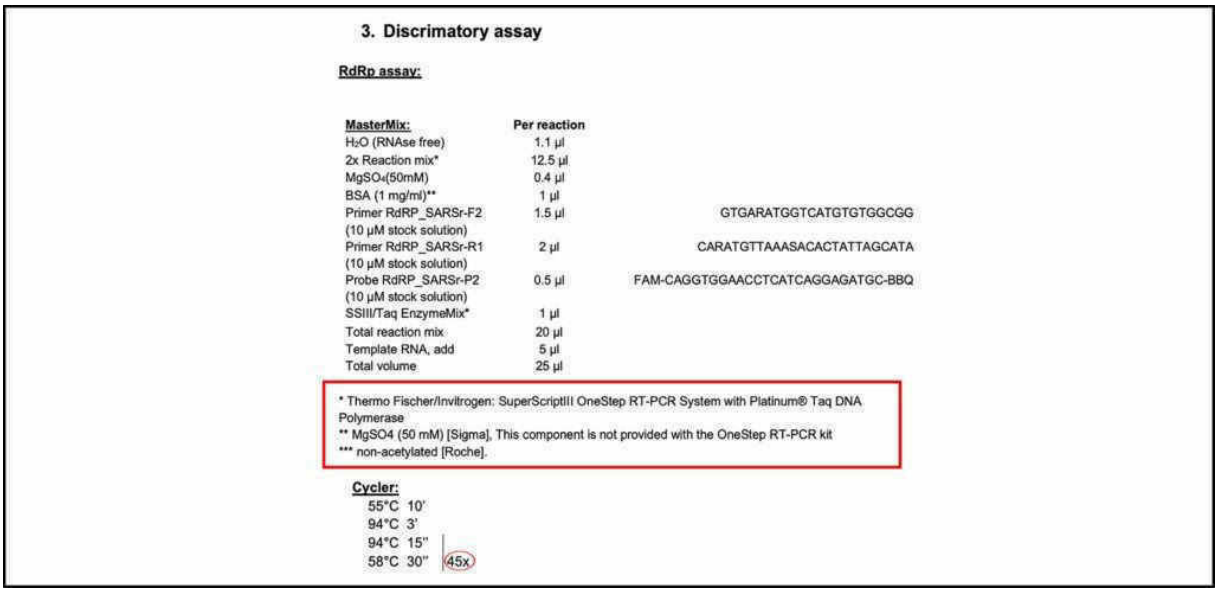
Citing Jaafar et al. 2020 [3]:

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

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

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

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



3. Discrimatory assay

RdRp assay:

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

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

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

4. Biomolecular validations

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

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

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

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

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

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

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

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

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

Third, the Corman-Drosten paper states:

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

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

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

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

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

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

6. Standard Operational Procedure (SOP) is not available

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

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

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

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

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

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

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

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

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

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

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

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

9. Authors as the editors

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

SUMMARY CATALOGUE OF ERRORS FOUND IN THE PAPER

The Corman-Drosten paper contains the following specific errors:

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

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

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

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

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

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

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

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

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

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

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

CONCLUSION

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

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

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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].

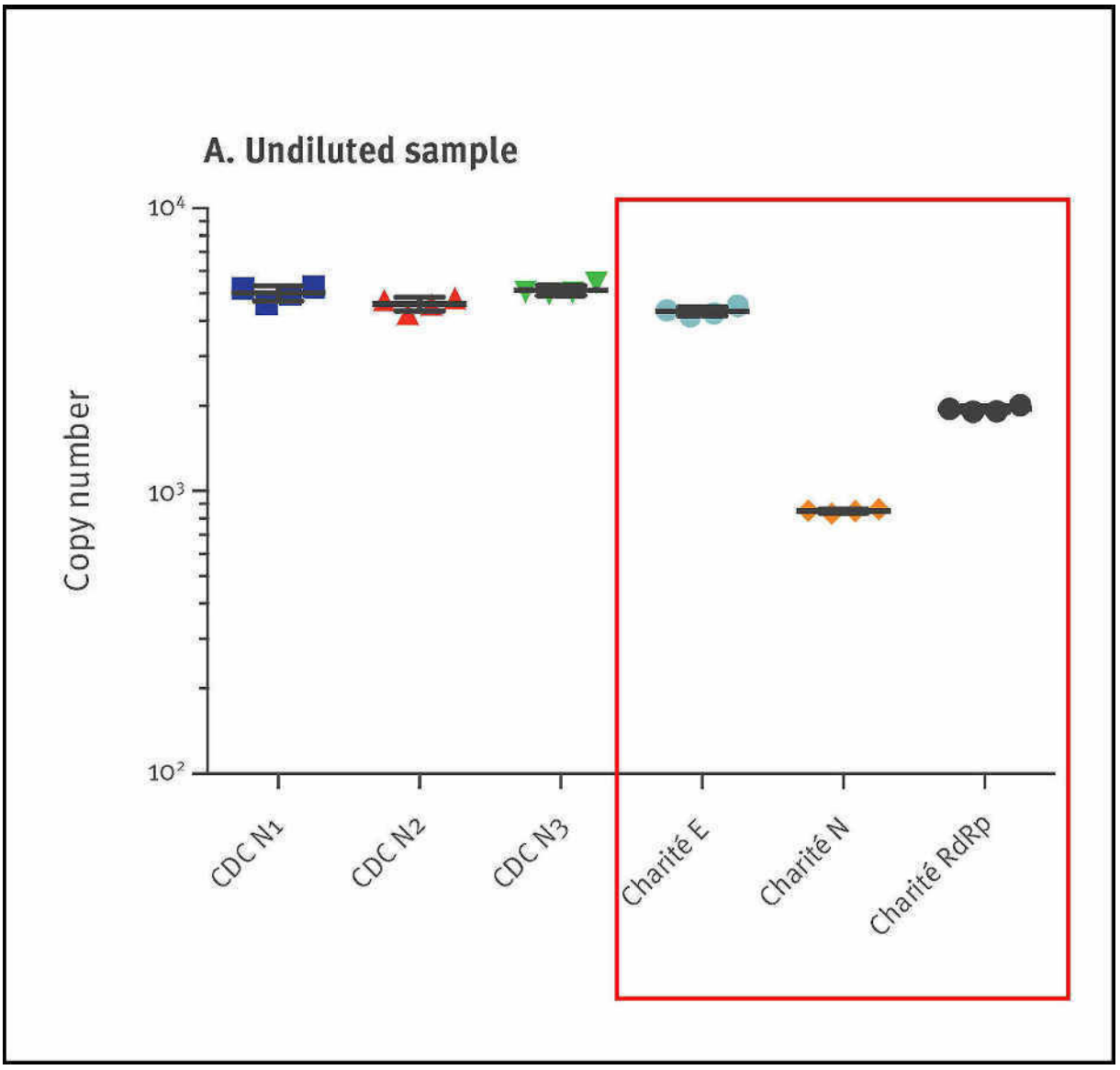
According to table 1 in the Muenchhoff *et al*. paper, the Corman-Drosten protocol components (primers, gene assays, etc.) are labeled and referred to as “Charité genes” and TIB-Molbiol is listed as the manufacturer of the corresponding primers/probes.

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).

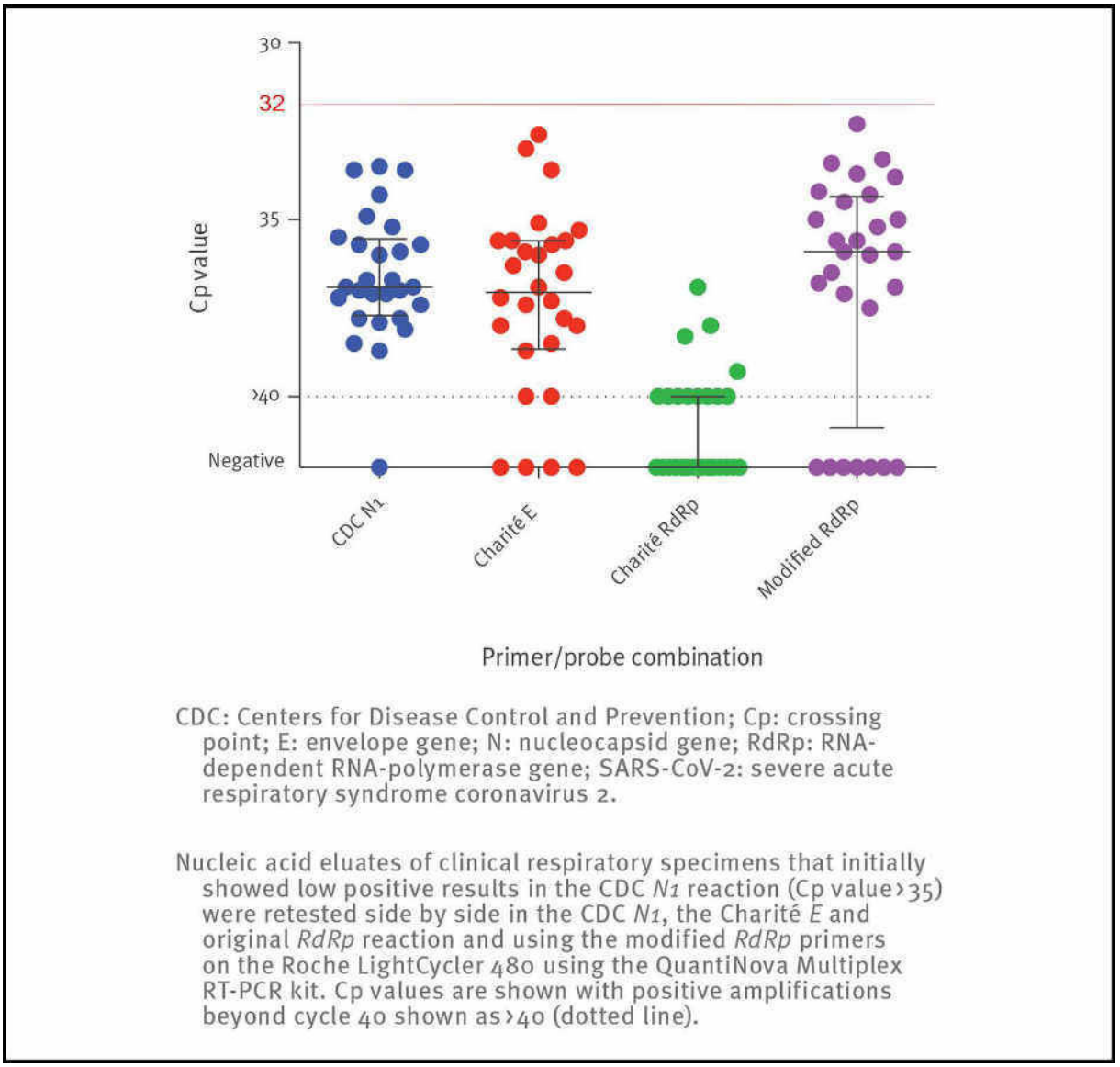
Based on the digital droplet PCR, the authors concluded that the “Charité E gene” primer pair performance is comparable with the “CDC N primer pairs”; both show similar sensitivity, but the N gene and the RdRp gene assays are **significantly less sensitive** with the positive RNA samples tested (Figure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

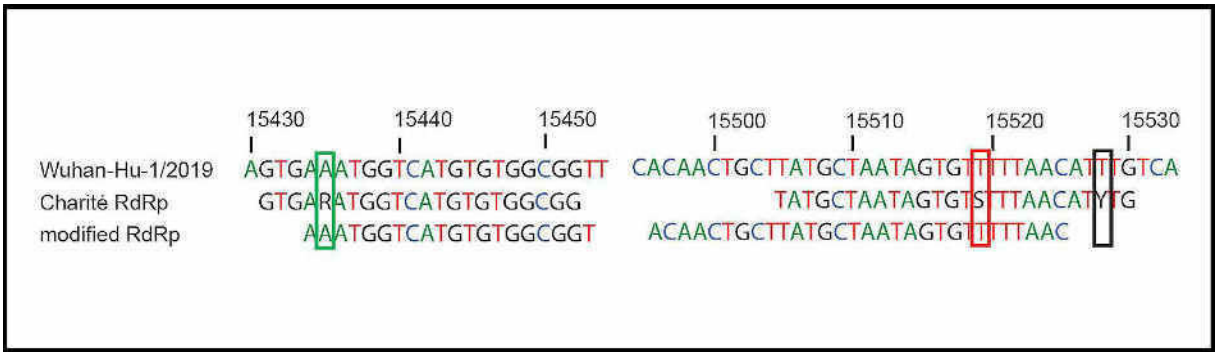
Nucleic acid eluates of clinical respiratory specimens that initially showed low positive results in the CDC *N1* reaction (Cp value›35) were retested side by side in the CDC *N1*, the Charité *E* and original *RdRp* reaction and using the modified *RdRp* primers on the Roche LightCycler 480 using the 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	GTTAAAAACACTATTAGCATAAGCAGTTG A	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]

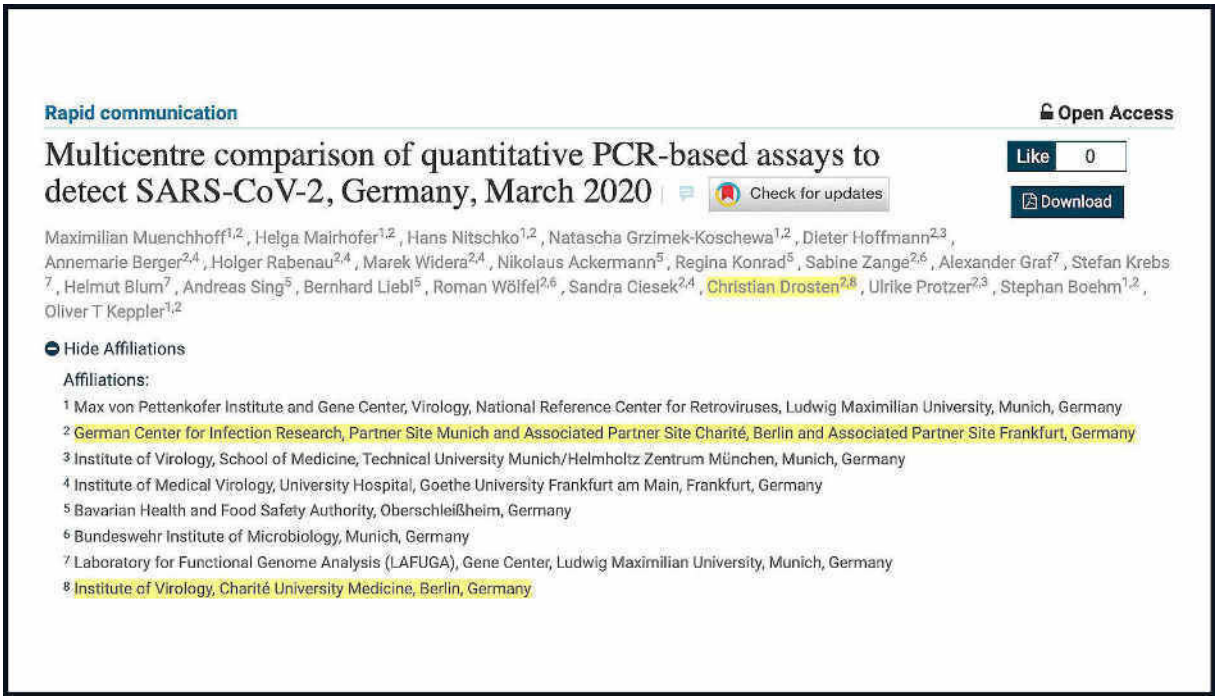
Further discussion of Muenchhoff *et al.*

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

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

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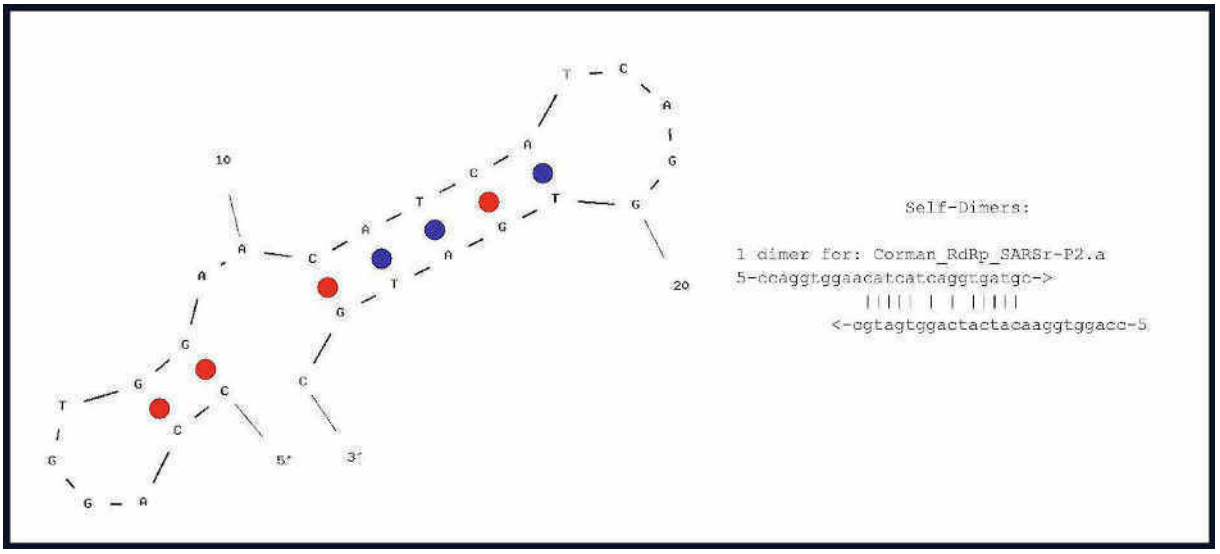
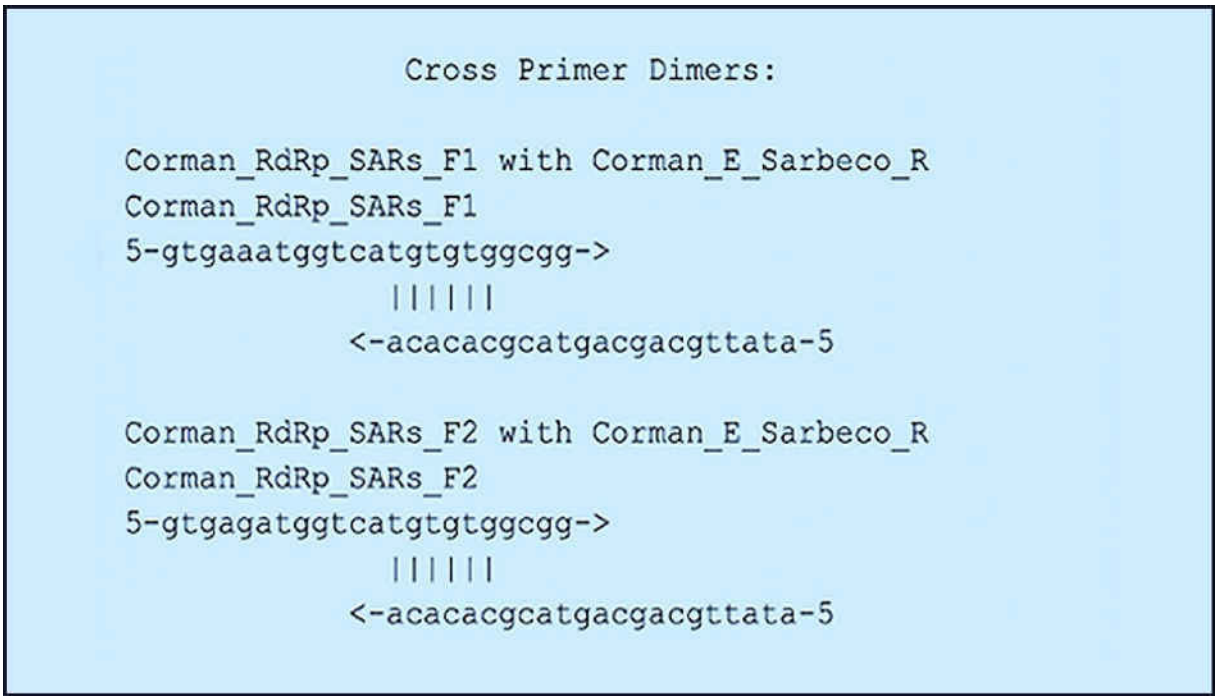
8 Institute of Virology, Charité University Medicine, Berlin, Germany

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.

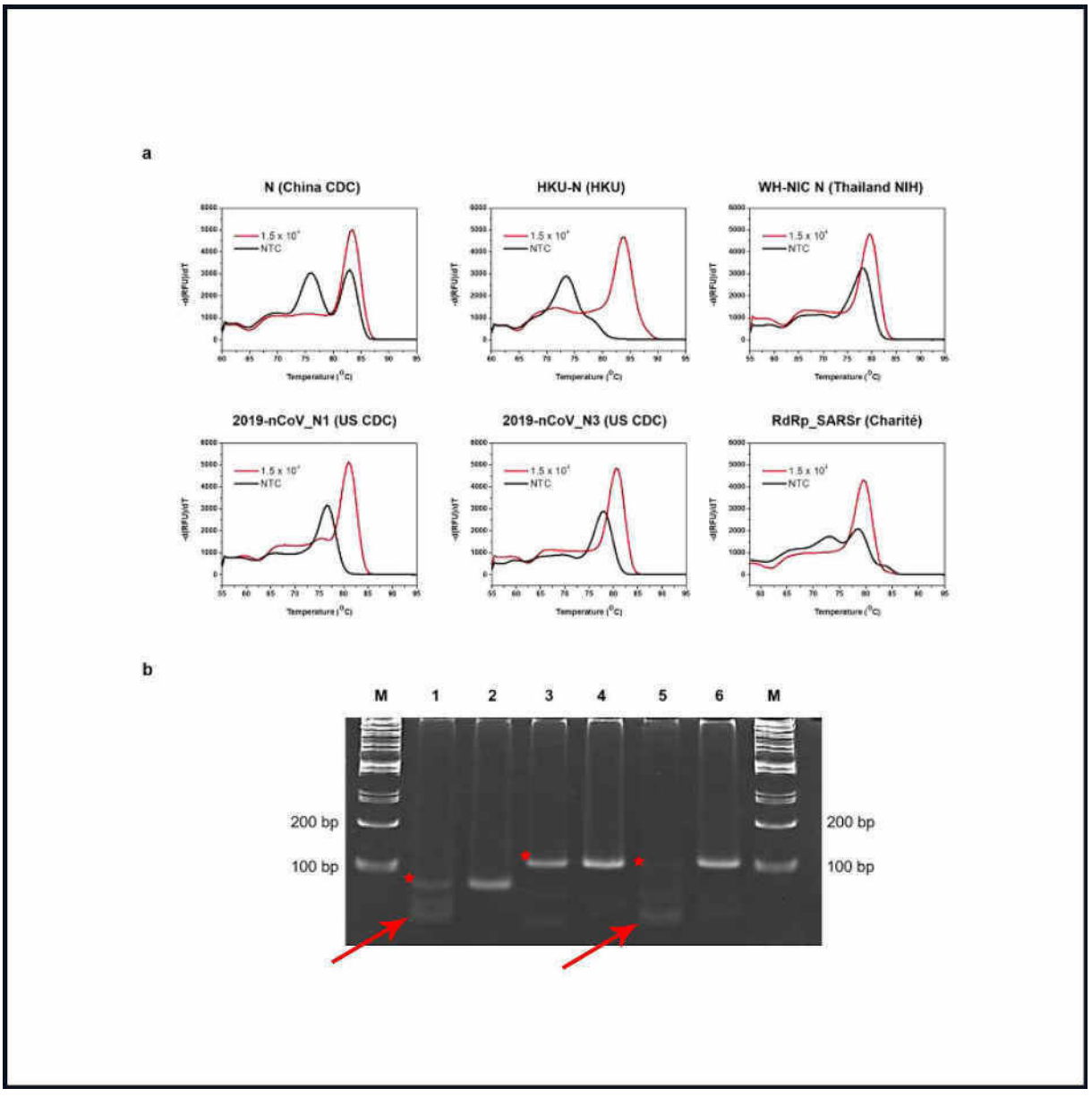
Jung *et al*. clearly refute a commonly voiced misconception, that reduced sensitivity of the Corman *et al.* protocol could only manifest itself with false negatives and should not create false positives.

"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]

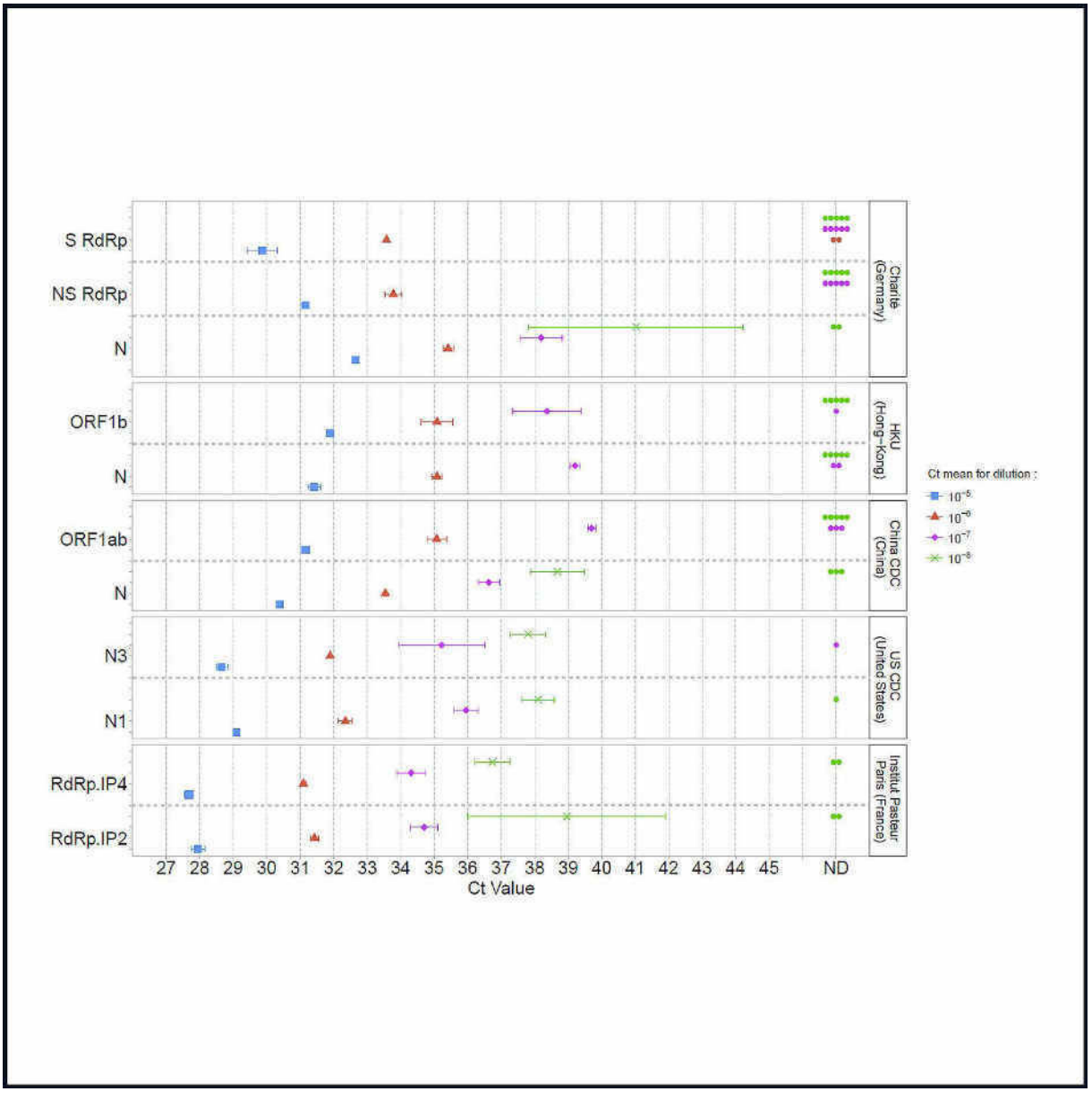
In theory, this should be a rare occurrence if labs are running singleplex assays without primer contamination, yet it is readily found in peer-reviewed literature with these exact assays and conditions by Etievant *et al*. Even with singleplex assays free of primer contamination, RdRp probe forms a 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].

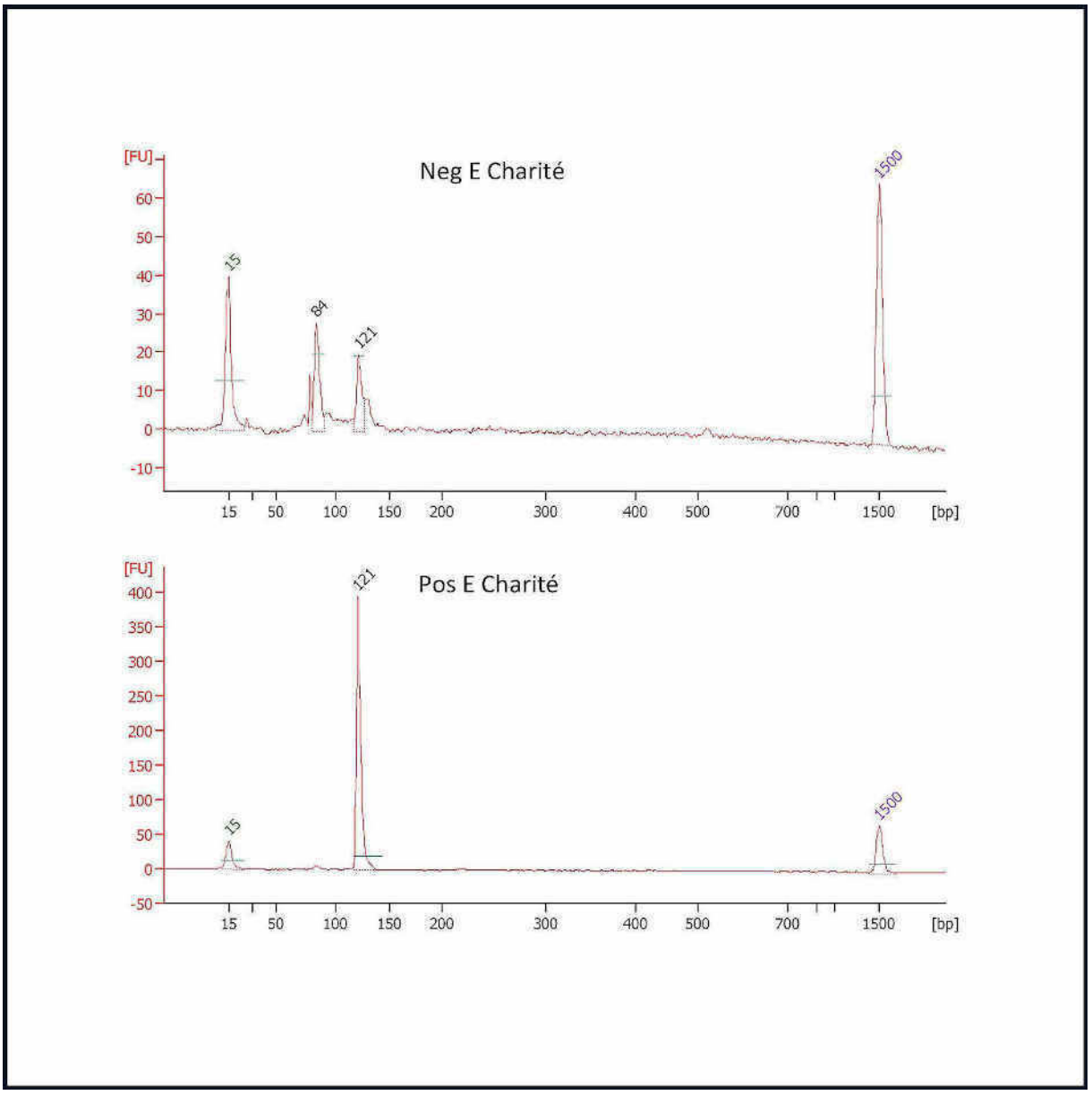
Figure 8 taken from Etievant *et al. (Figure 1)*:
Mean Ct values and standard deviations obtained using five PCR-based methods for SARSCoV-2 detection. Serial dilutions of SARS-CoV-2 cell culture supernatants were used and are represented by a single color (10−5 blue, 10−6 red, 10−7 pink, 10−8 green). 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.*

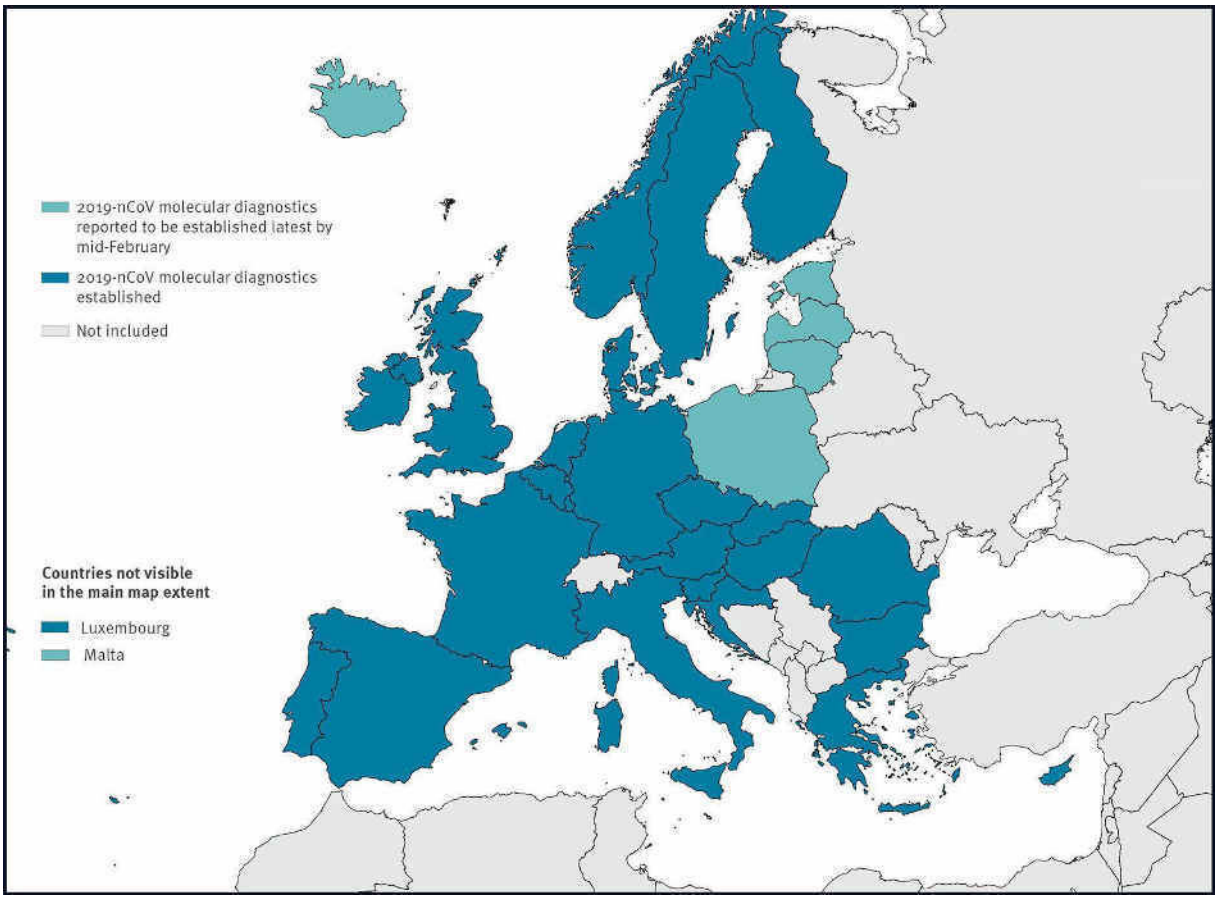
Gand *et al*. [10] notes that the Charité primers were the most widely used in Europe in the spring of 2020, referencing Reusken *et al*. published at the end of January 2020 at Eurosurveillance:

"*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.

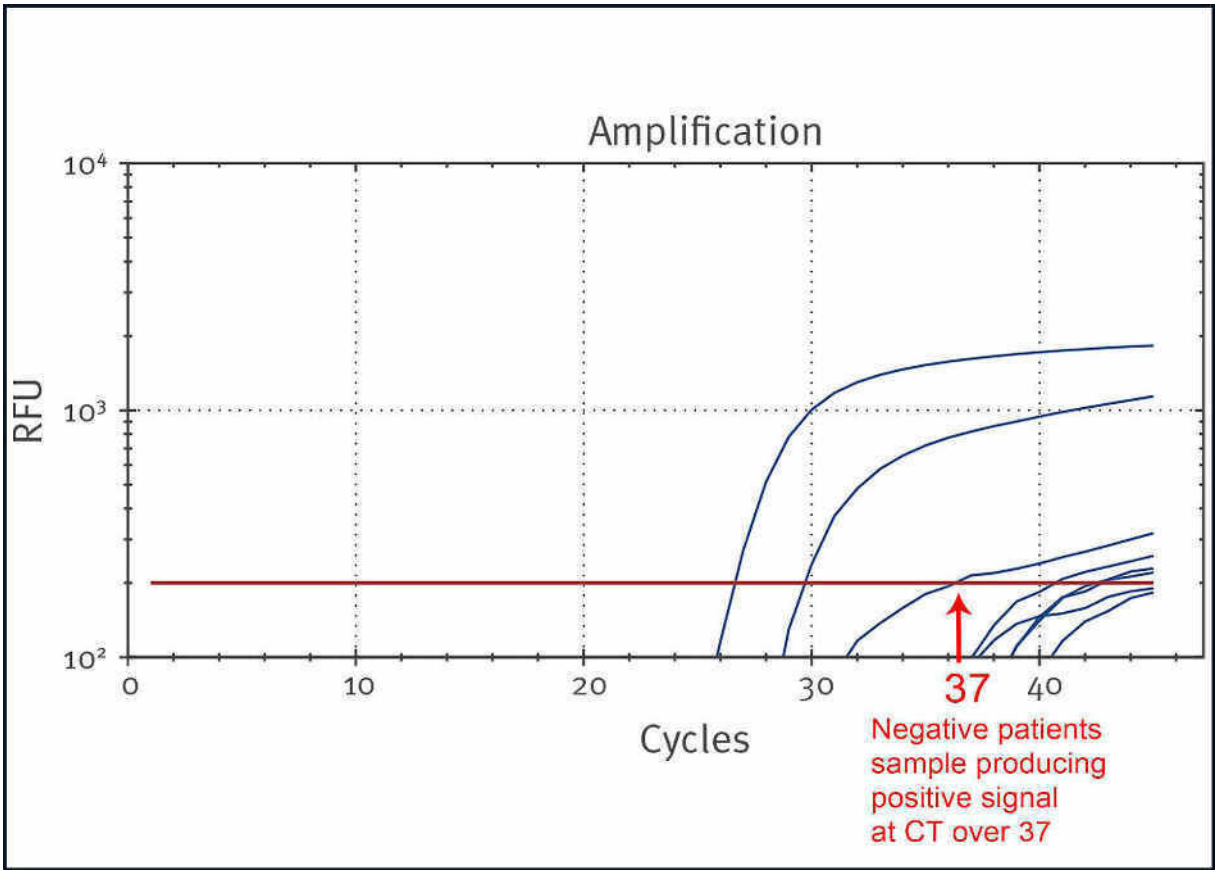
*"Interestingly, for Assay_2_RdRp-P2, similar false-positive results **as obtained in our in silico study** were obtained in the wet lab by Chan and colleagues, who detected SARS-CoV when using the probe P2 targeting the RdRp gene that is considered strictly specific to SARS-CoV-2. This indicates that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

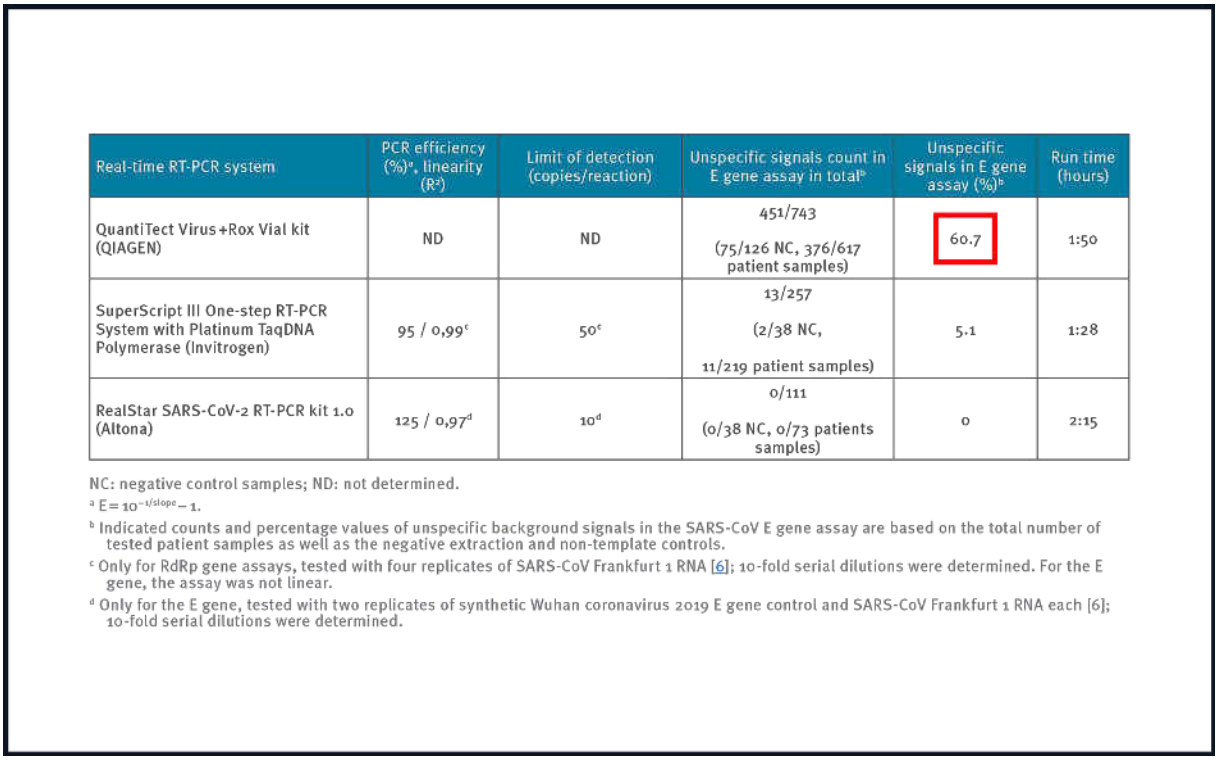
Figure 11: taken from Konrad et al (Figure 2):
Example image of real-time RT-PCR curves of the gene assay with unspecific signals at late cycles, Bavaria, February 2020



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).*

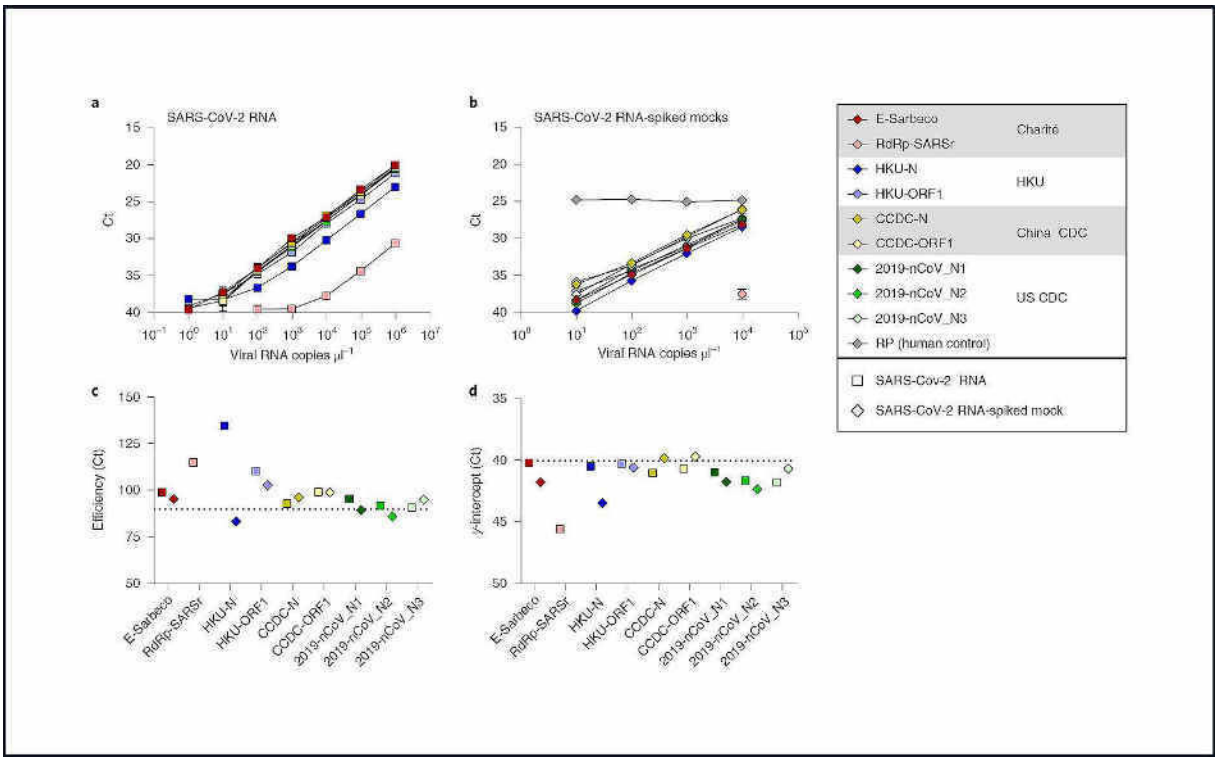
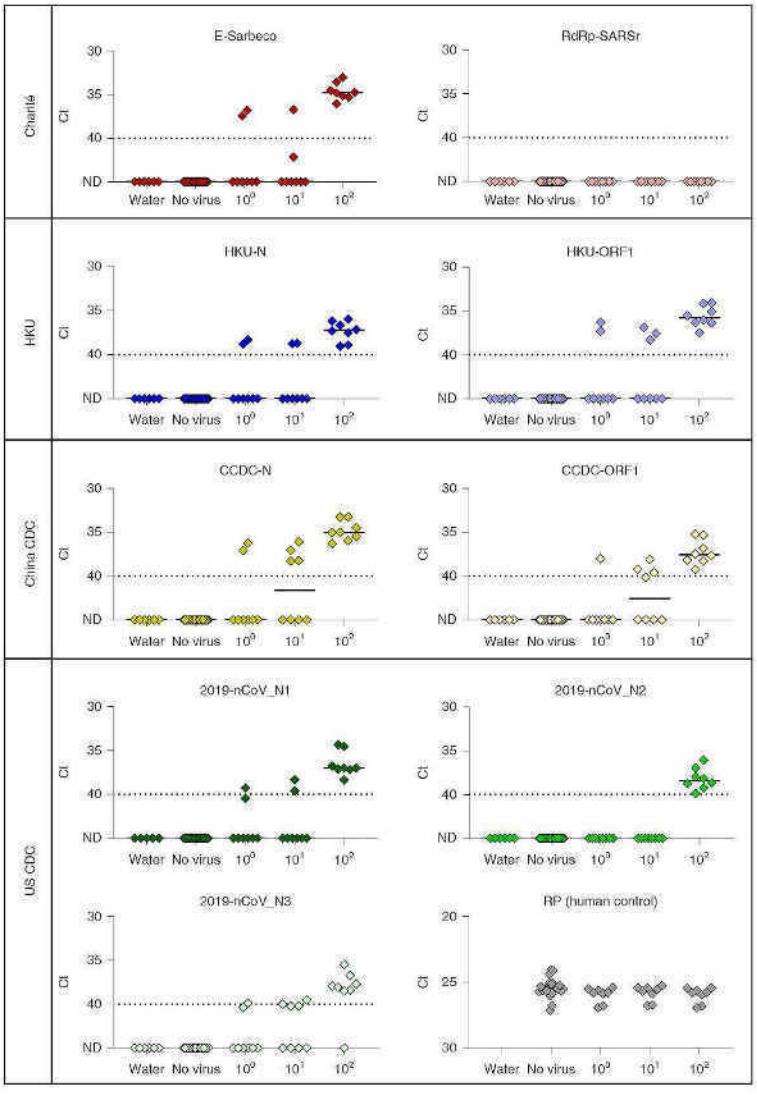


Figure 16 taken from Vogels *et al*. (Fig.2):

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)

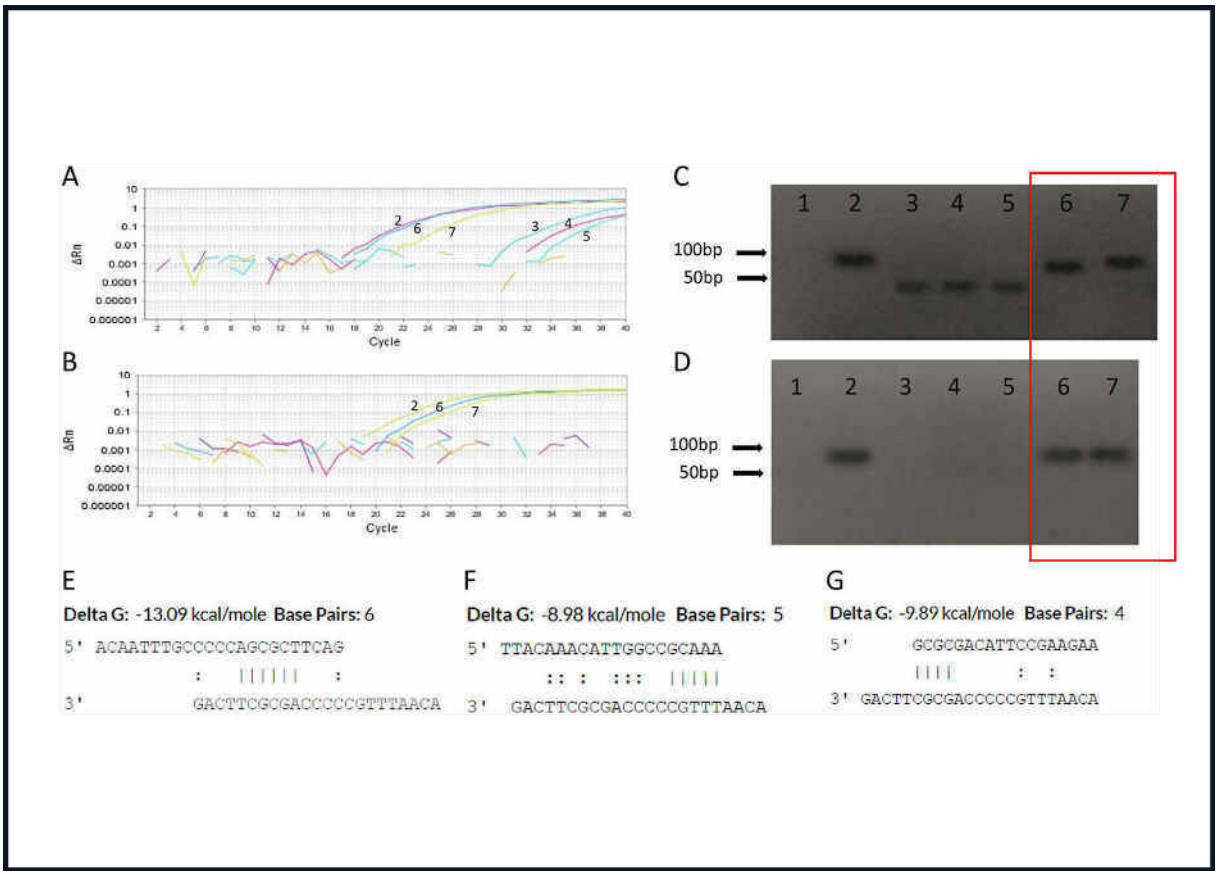
Note their specific comment that speaks of fluorogenic probe-based assays typically not generating signals but with these promiscuous primers they generate false positive signals**.**

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:

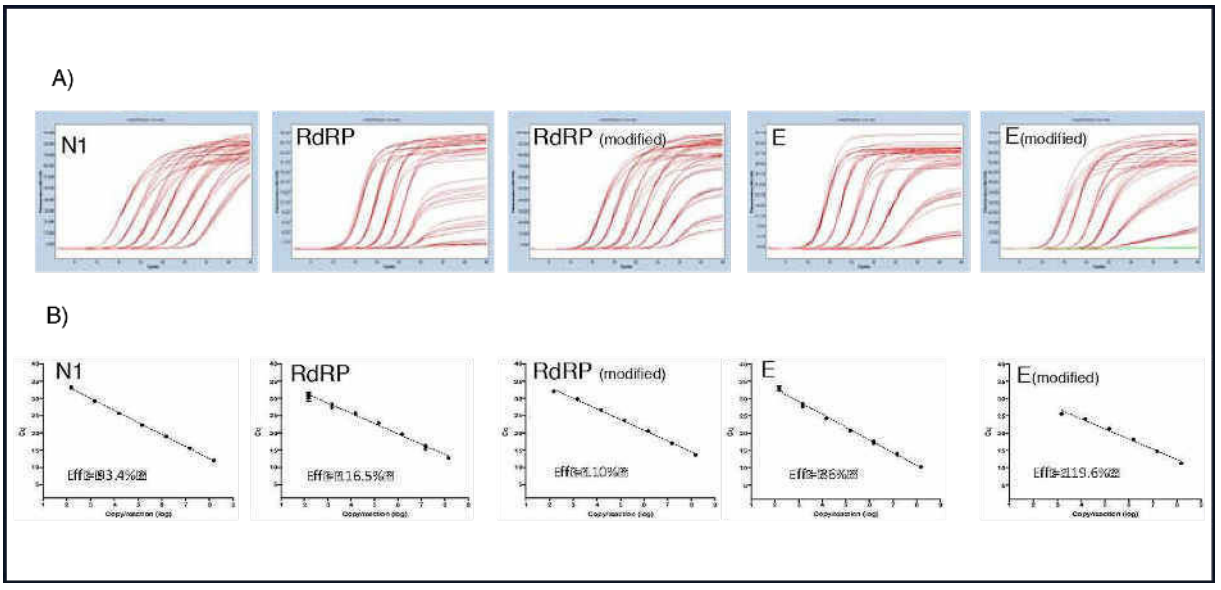
"*Future studies should also include the comparison of in-house RdRP RT-PCR with commercial RT-PCR. Indeed, this comparison was not achieved as the RdRP RT-PCR needed further optimization based on recent publication that elucidated the reason of the limited sensitivity as the difference in the melting 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:

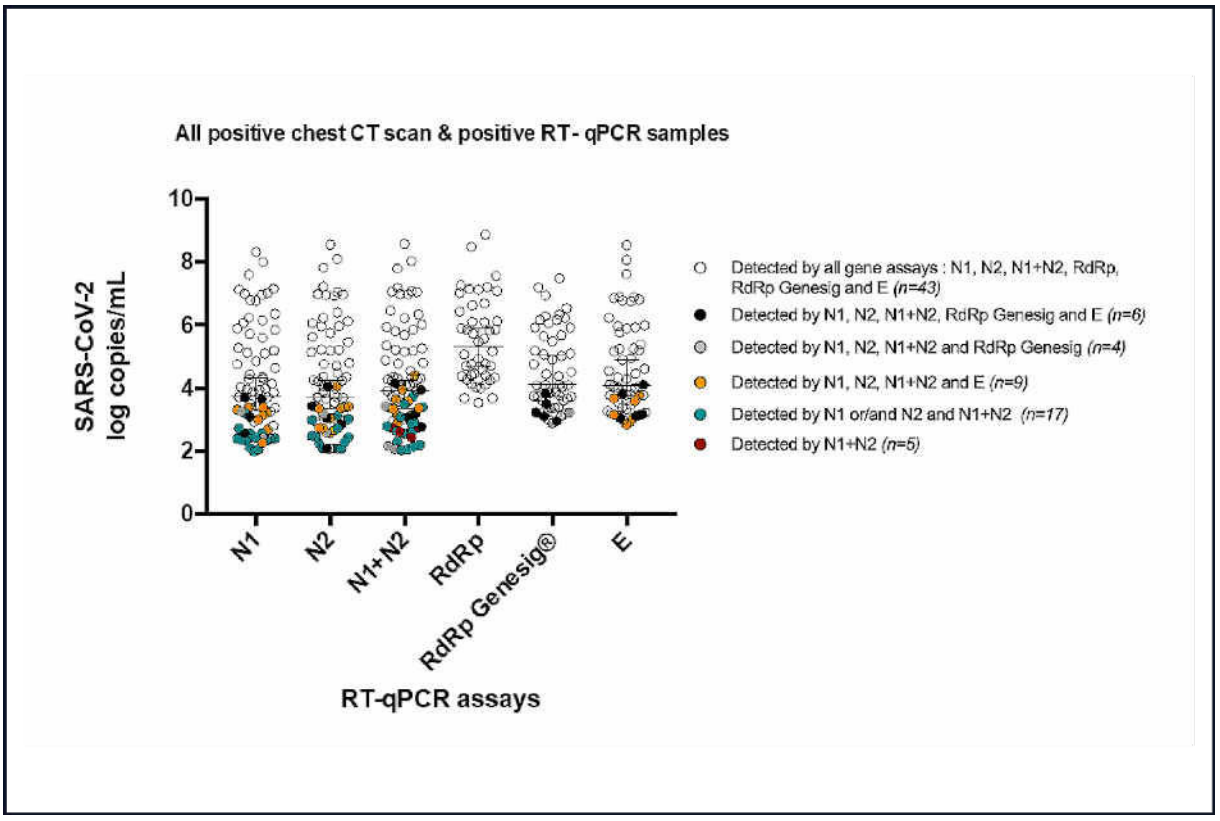
"Incorrect degenerate base S at position 12 that binds with G or C while all SARS-CoV-2 analyzed sequences encoded for a T at this position [...]. This mismatch would not be derived from a new variant but rather due to the initial oligonucleotide design allowing to amplify SARS-CoV, bat-SARS-related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

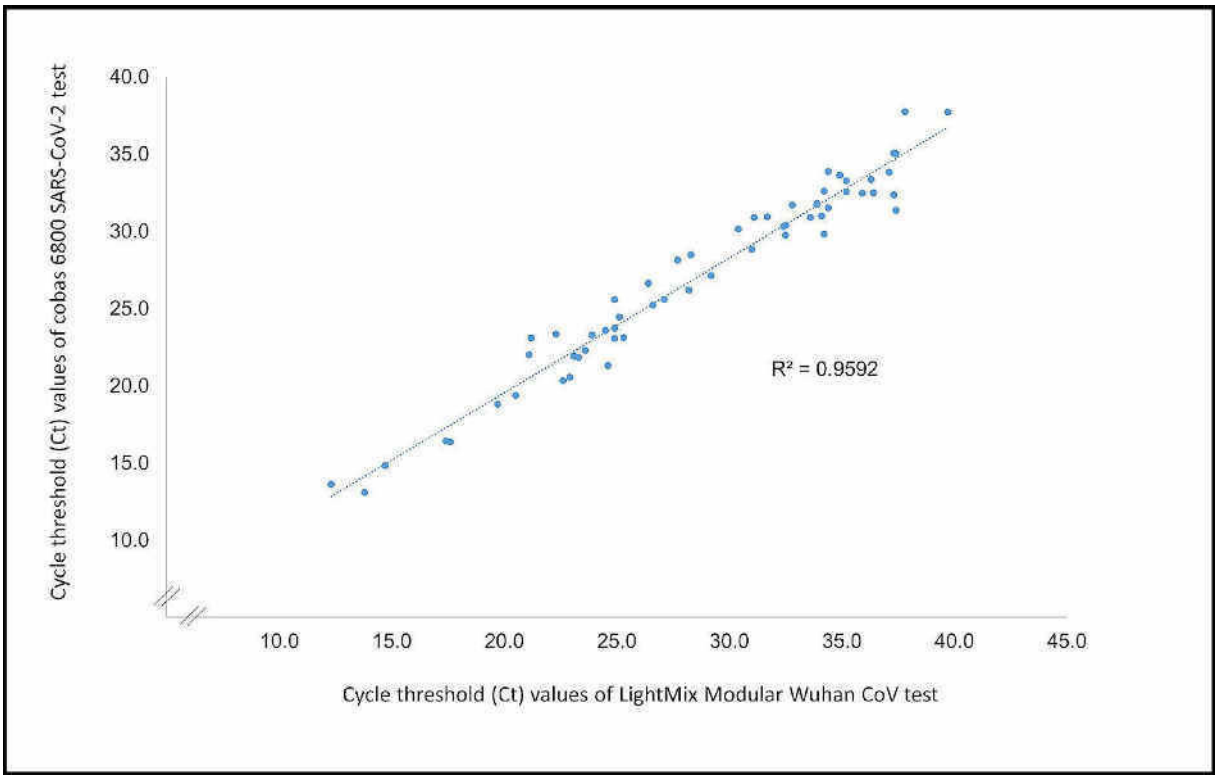
Second test (prospective comparison).
1 of 502 samples was excluded from analysis due to invalid cobas results
2 of 438 samples which were negative by LightMix were found positive by cobas

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.

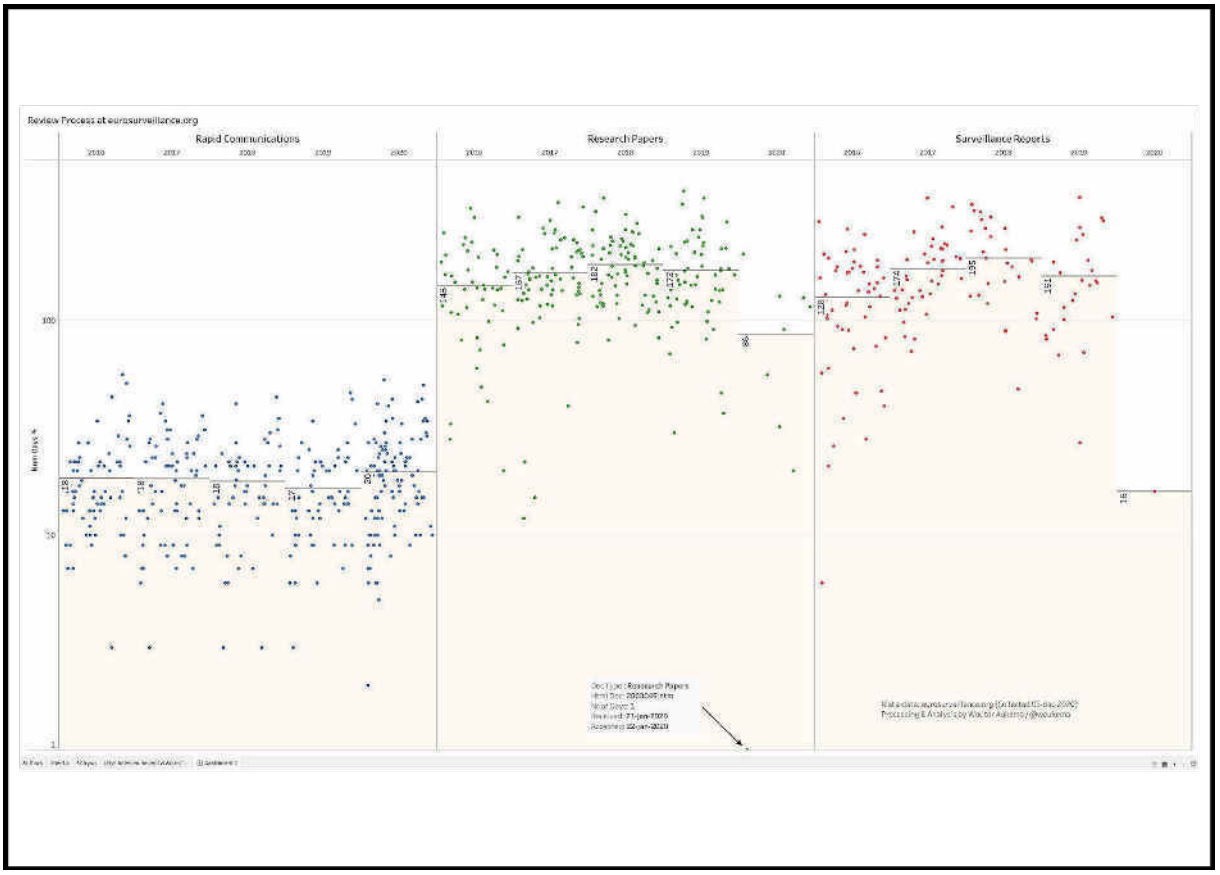
This six-page document aims to make these findings reproducible and verifiable by offering step by step instructions.

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

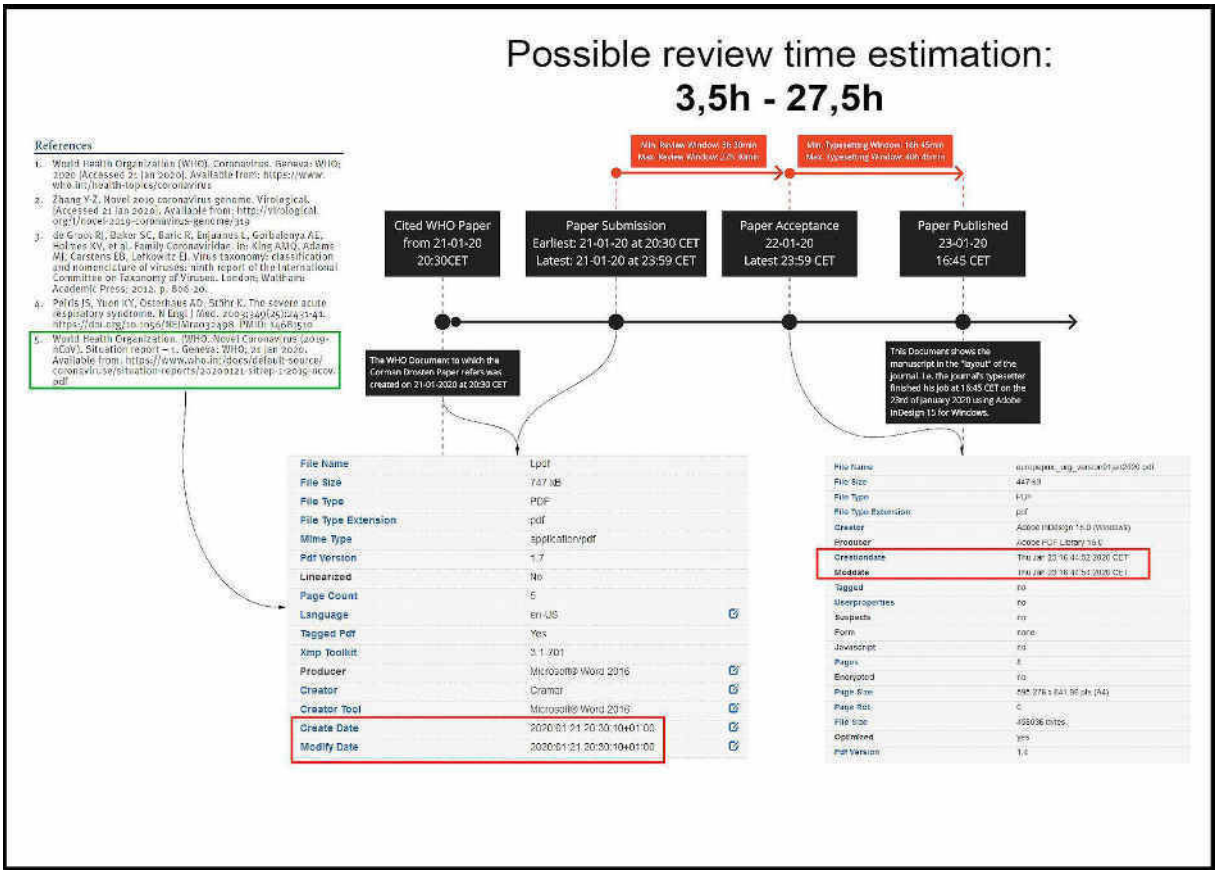
GenExpress and serves as scientific advisor for Tib-Molbiol. Given the unbelievably short review time, we have to further conclude:

- 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.

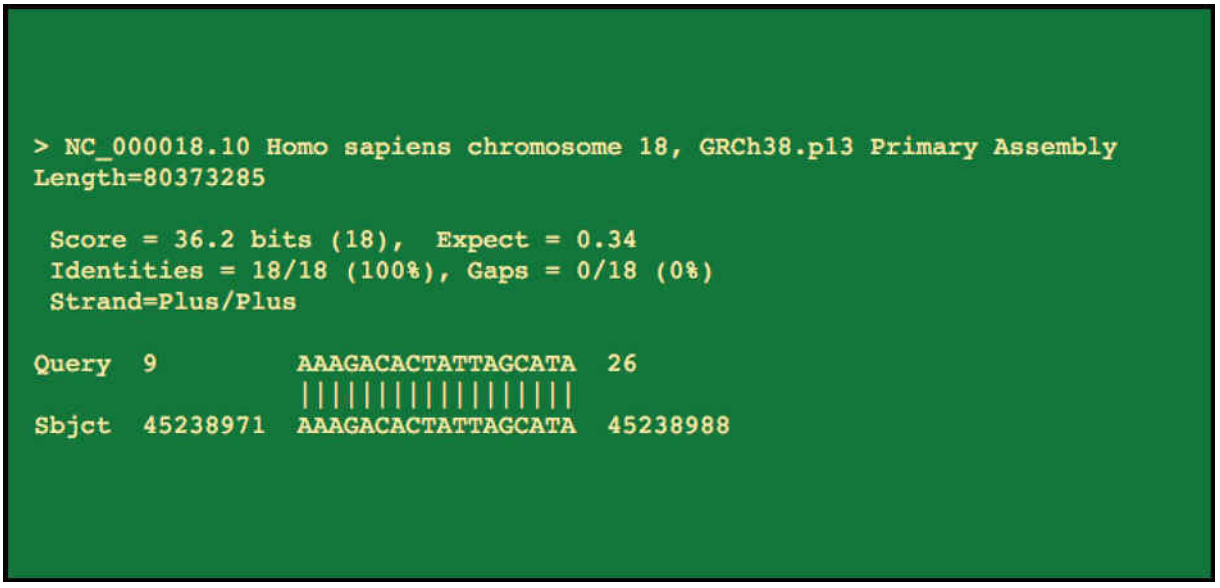
We have included a BLAST analysis of the Charité primers against the Human Genome (GRCh38.p13). There are several significant homologies but none that have both primer and probes in close proximity. While these off-target homologies are not catastrophic for assay performance, they do demonstrate the 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].

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Supplementary Material

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Additional Proof Readers:
Howard Steen
Paul Gregory
Peter Davies

Gmail

This message has been automatically translated: Lithuanian -> English.

Christine Massey <cmssyc@gmail.com>

Fw: ON THE FORWARDING OF THE REQUEST BY ARTURAS BARTAŠIUS

Arturas Bartasius <bartasius.arturas@yahoo.com> **Wed, Sep 22, 2021 at 11:10 PM**
Reply-To: Arturas Bartasius <bartasius.arturas@yahoo.com>
To: "cmssyc@gmail.com" <cmssyc@gmail.com>

Sent from Yahoo Mail on Android

> ----- Forwarded Message -----
> **From:** " kontora@nvsplt.onmicrosoft.com " < kontora@nvsplt.onmicrosoft.com >
> **To:** " bartasius.arturas@yahoo.com " < bartasius.arturas@yahoo.com >
> **Sent:** Wed, Sep 22, 2021 at 14:48
> **Subject:** INQUIRY FOR REFERRAL ARTŪRO Bartašius
>
> We send you the National Public Health Laboratory 2021-09-22 document no. S-1076.
>
> *This is an automated notification, please email email address not to reply.*
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>
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8 attachments — Download all attachments

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NACIONALINĖ VISUOMENĖS SVEIKATOS PRIEŽIŪROS LABORATORIJA

Biudžetinė įstaiga, Žolyno g. 36, LT-10210 Vilnius, tel. (8 5) 270 9229, faks. (8 5) 210 4848
el. p. nvspl@nvspl.lt, www.nvspl.lt
Duomenys kaupiami ir saugomi Juridinių asmenų registre, kodas 195551983

Artūrui Bartašiui
El. p. bartasius.arturas@yahoo.com

2021-09-
Į 2021-09-17

Nr.
Nr. (08 16.1.17 Mr)2-113823

Kopija
Nacionaliniam visuomenės sveikatos centrui prie
Sveikatos apsaugos ministerijos

DĖL ARTŪRO BARTAŠIAUS PAKLAUSIMO PERSIUNTIMO

Nacionalinė visuomenės sveikatos priežiūros laboratorija (toliau – NVSPL) išnagrinėjusi piliečio Artūro Bartašiaus paklausimą teikia SARS-CoV-2 nustatymui polimerazės grandininės reakcijos metodu šiuo metu naudojamų reagentų aprašus ir instrukcijas. Šiuos reagentus naudoja NVSPL ir kitos SARS-CoV-2 PGR tyrimus atliekančios Lietuvos laboratorijos.

Atkreipiame dėmesį, kad SARS-CoV-2 viruso išskyrimas ląstelių kultūrose, elektroninės mikroskopijos tyrimas bei viruso išgryninimas (filtravimas, ultracentrifugavimas, chromatografija) NVSPL neatliekamas.

Pridedama:
1.TaqPath COVID-19 CE-IVD RT-PCR Kit, 1egz.
2.Allplex SARS-CoV-2 Assay, 1egz.

Direktoriaus pavaduotoja,

laikinai einanti direktoriaus pareigas Rosita Marija Balčienė

Svajūnė Muralytė, tel. +37067180340, el. p. svajune.muralyte@nvspl.lt

(AUTO-TRANSLATION)

NATIONAL PUBLIC HEALTH CARE LABORATORY

Budgetary institution, Žolyno str. 36, LT-10210 Vilnius, tel. (8 5) 270 9229, fax. (8 5) 210 4848
el. p. nvspl@nvspl.lt, www.nvspl.lt
Data are collected and stored in the Register of Legal Entities, code 195551983

Artūras Bartašis
El. p. bartasius.arturas@yahoo.com

2021-09-
Į 2021-09-17
Nr.
Nr. (08 16.1.17 Mr)2-
113823

Copy
National Center for Public Health at
Ministry of Health

ON THE FORWARDING OF THE REQUEST BY ARTŪR BARTAŠIS

National Public Health Laboratory (hereinafter - NSPL) after examining the request of the citizen Artūras Bartašius, provides a polymerase assay for the determination of SARS-CoV-2 descriptions and instructions for the reagents currently used in the chain reaction method. These reagents used by NVSPL and other Lithuanian laboratories performing SARS-CoV-2 PCR tests.

Note that isolation of SARS-CoV-2 virus in cell cultures, electron microscopy and virus purification (filtration, ultracentrifugation, chromatography) NVSPL is not performed.

Attached:
1.TaqPath COVID-19 CE-IVD RT-PCR Kit, 1gz.
2. Allplex SARS-CoV-2 Assay, 1gz.

Deputy Director,
acting Director Rosita Marija Balčienė

Svajūnė Muralytė, tel. +37067180340, el. p. svajune.muralyte@nvspl.lt

Translated sections:

FOI request

1) With this FOI request I would like you to provide me with the following information: A scientific research (no review document) that shows that the virus SARS-CoV-2 exists. The research should comply with the “state-of-the-art” isolation of the virus and prove that the coronavirus has a unique structure and consists of a unique viral genetic substance / genetic sequence. Control experiments must have been executed and documented in accordance with the scientific guidelines, to prove that non-typical cellcomponents have not been misinterpreted as viral components.

Response to FOI request

2) The obligation to publish documents according to the FOI act does not apply to information that is already public. The requested information has already been published and can be found on different websites such as:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7159086/ and
https://pubmed.ncbi.nlm.nih.gov/31978945

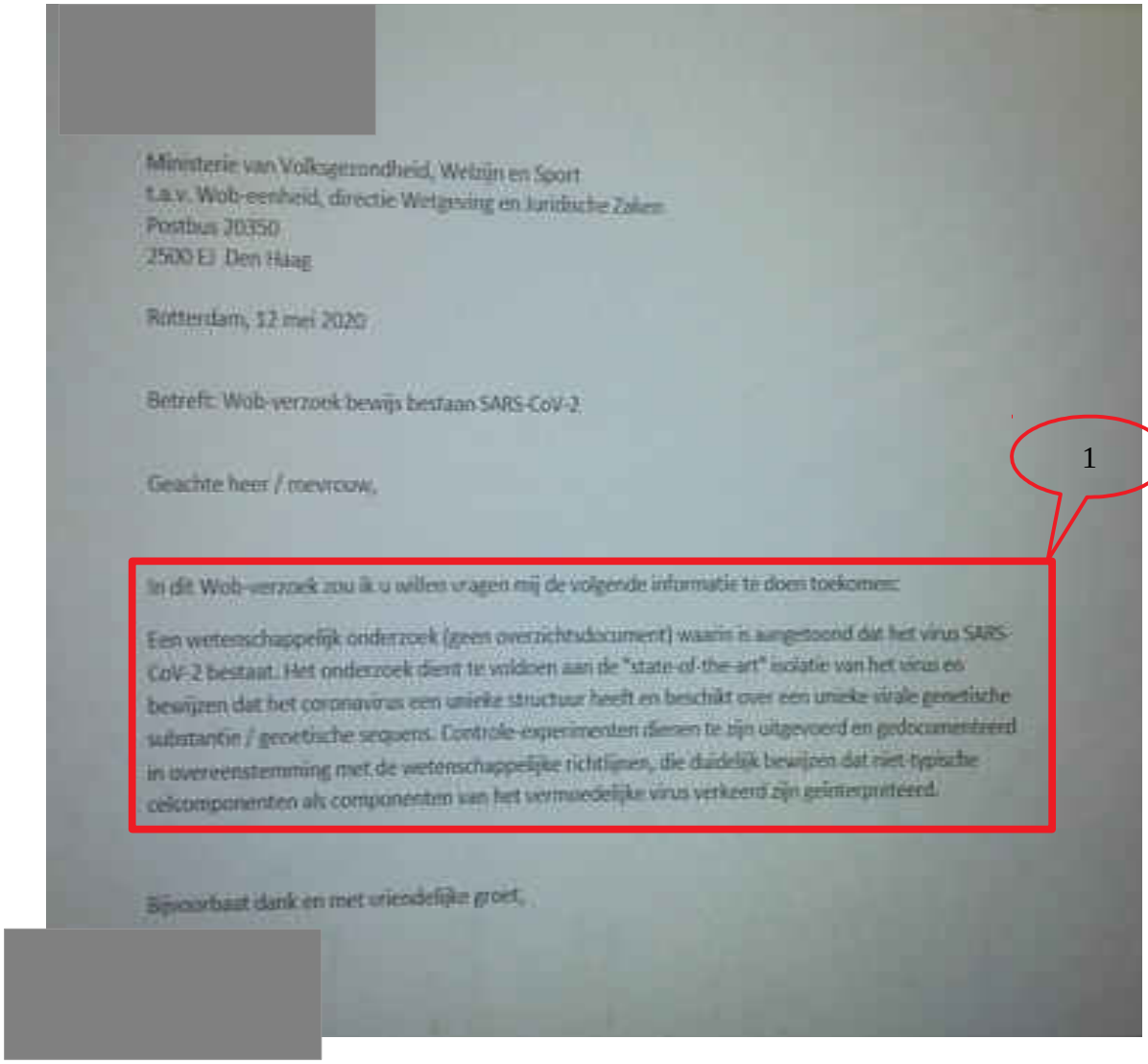
Objection letter

3) However, none of the 2 scientific publications provide proof of the existence of SARS-CoV-2. There is no report of purification of the virus and control experiments have not been executed.

Response to objection letter

4) Following your request I have inquired with the RIVM. From this inquiry it follows that the RIVM relies on public information resources, of which the two in my letter of the 2nd of juli 2020 are examples. According to the RIVM these two publications provide proof of the existence of the virus SARS-CoV-2.

FOI request proof of the existence of sars-cov-2



[redacted]

Ministerie van Volksgezondheid, Welzijn en Sport
t.a.v. Wob-eenheid, directie Wetgeving en Juridische Zaken
Postbus 20350
2500 EJ Den Haag

Rotterdam, 12 mei 2020

Betreft: Wob-verzoek bewijs bestaan SARS-CoV-2

Geachte heer / mevrouw,

1

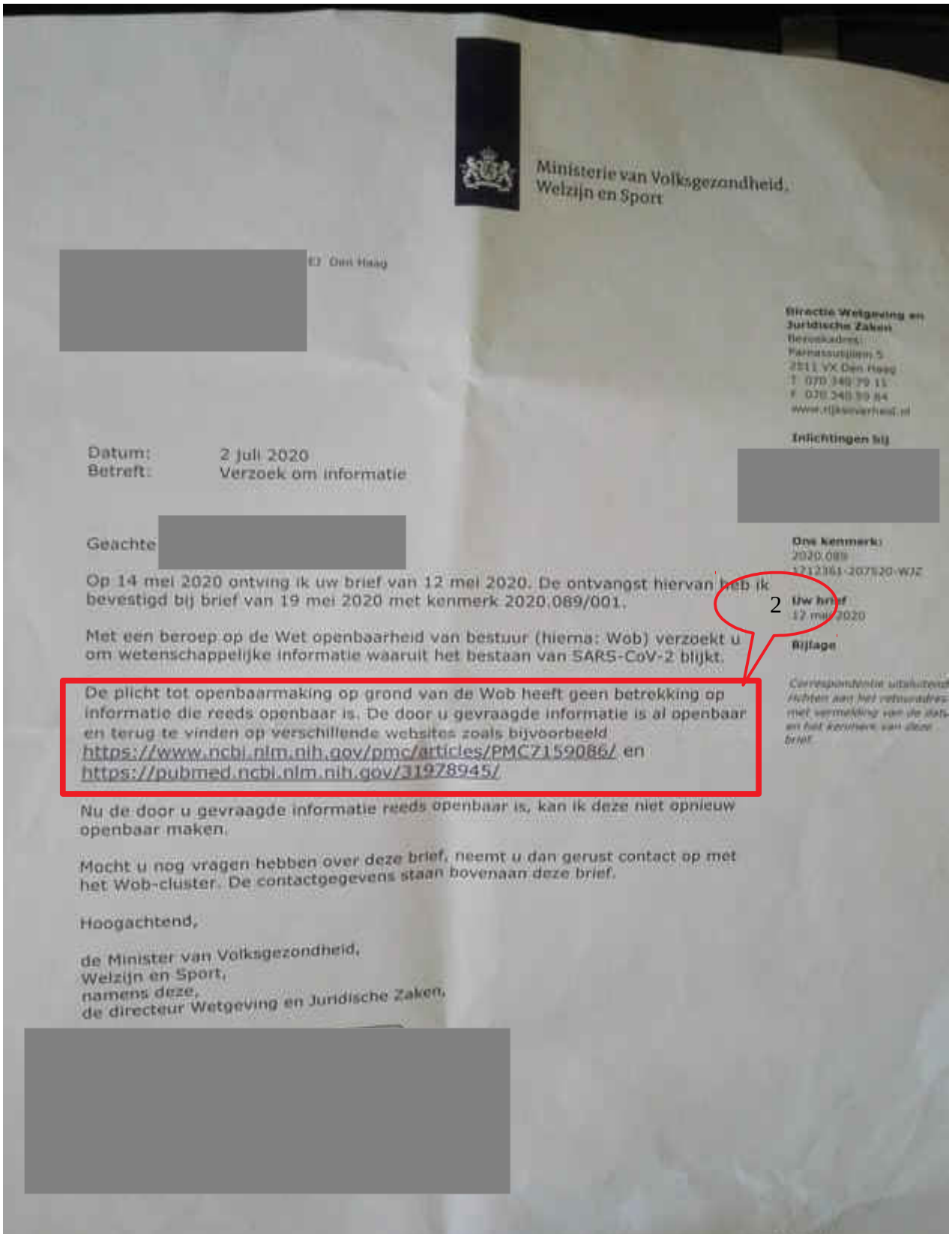
In dit Wob-verzoek zou ik u willen vragen mij de volgende informatie te doen toekomen:

Een wetenschappelijk onderzoek (geen overzichtsdocument) waarin is aangetoond dat het virus SARS-CoV-2 bestaat. Het onderzoek dient te voldoen aan de "state-of-the-art" isolatie van het virus en bewijzen dat het coronavirus een unieke structuur heeft en beschikt over een unieke virale genetische substantie / genetische sequens. Controle-experimenten dienen te zijn uitgevoerd en gedocumenteerd in overeenstemming met de wetenschappelijke richtlijnen, die duidelijk bewijzen dat niet-typische celcomponenten als componenten van het vermoedelijke virus verkeerd zijn geïnterpreteerd.

Bijvoorbaat dank en met vriendelijke groet,

[redacted]

Response to FOI



Ministerie van Volksgezondheid,
Welzijn en Sport

[redacted] Den Haag

Directie Wetgeving en Juridische Zaken
Bezoekadres:
Parnassusplein 5
2511 VX Den Haag
T 070 340 79 11
F 070 340 79 84
www.rijksoverheid.nl

Inlichtingen bij
[redacted]

Datum: 2 juli 2020
Betreft: Verzoek om informatie

Ons kenmerk:
2020.089
1212361-207520-WJZ

Uw brief
12 mei 2020

Bijlage

Correspondentie uitsluitend richten aan het retouradres met vermelding van de datum en het kenmerk van deze brief.

Geachte [redacted]

Op 14 mei 2020 ontving ik uw brief van 12 mei 2020. De ontvangst hiervan heb ik bevestigd bij brief van 19 mei 2020 met kenmerk 2020.089/001.

Met een beroep op de Wet openbaarheid van bestuur (hierna: Wob) verzoekt u om wetenschappelijke informatie waaruit het bestaan van SARS-CoV-2 blijkt.

2

De plicht tot openbaarmaking op grond van de Wob heeft geen betrekking op informatie die reeds openbaar is. De door u gevraagde informatie is al openbaar en terug te vinden op verschillende websites zoals bijvoorbeeld https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7159086/ en https://pubmed.ncbi.nlm.nih.gov/31978945/

Nu de door u gevraagde informatie reeds openbaar is, kan ik deze niet opnieuw openbaar maken.

Mocht u nog vragen hebben over deze brief, neemt u dan gerust contact op met het Wob-cluster. De contactgegevens staan bovenaan deze brief.

Hoogachtend,

de Minister van Volksgezondheid,
Welzijn en Sport,
namens deze,
de directeur Wetgeving en Juridische Zaken,

[redacted]

Objection letter

[redacted]

20-7-2020 08:37

Bezwaarschrift

[redacted]

Beste heer/mevrouw [redacted]

Met dit schrijven dien ik een bezwaarschrift in met betrekking tot uw brief met kenmerk 2020.089 1712361-207520-WJZ.

De brief is een antwoord op mijn wob-verzoek van 12 mei 2020. In mijn verzoek vraag ik naar een document waaruit blijkt dat SARS-CoV-2 bestaat.

In het antwoord geeft de heer/mevrouw [redacted] aan dat de wob geen betrekking heeft tot informatie die reeds openbaar is en verwijst naar 2 wetenschappelijke publicaties waarin de informatie waarnaar ik heb gevraagd reeds openbaar zou zijn gemaakt.

3

Echter, in geen van de twee wetenschappelijke publicaties is sprake van een bewijs voor het bestaan van SARS-CoV-2. Er heeft namelijk geen purificatie van een virus plaatsgevonden en zijn er geen controle experimenten uitgevoerd.

U heeft dus niet voldaan aan mijn wob verzoek. Ik verzoek u daarom om mij alsnog de gevraagde informatie toe te sturen. Indien u niet over een document beschikt dat bewijst dat SARS-CoV-2 bestaat, wat ook mogelijk is, kunt u mij dat ook bevestigen.

Ook, zou ik u er op willen wijzen dat in de wet openbaarheid bestuur de volgende definitie is omschreven van een document: een bij een bestuursorgaan berustend schriftelijk stuk of ander materiaal dat gegevens bevat; Aangezien in deze definitie niet specifiek vermeld dat het document al dan niet egens anders is gepubliceerd kan het dus ook gaan om een document dat al eerder is gepubliceerd, maar bij u aanwezig is. Een verwijzing naar een peer-reviewed wetenschappelijk tijdschrift is dan ook mogelijk, allen als die verwijzing een antwoord is op mijn vraag.

Met vriendelijke groet,

[redacted]

Ministerie van Volksgezondheid, Welzijn en Sport

Retouradres Postbus 20350 2500 EJ Den Haag

[redacted]

Alleen per e-mail: [redacted]

Secretaris Generaal / plv. Secretaris Generaal
Directie Wetgeving en Juridische Zaken
Cluster 1

Bezoekadres:
Parnassusplein 5
2511 VX Den Haag
T 070 340 79 11
F 070 340 78 34

www.rijksoverheid.nl

Inlichtingen bij
[redacted]

Kenmerk
DWJZ-2020000372
1737238-209457-WJZ

Bijlage(n)
-

Correspondentie uitsluitend richten aan het retouradres met vermelding van de datum en het kenmerk van deze brief.

Datum – 4 SEP. 2020
Betreft Beslissing op bezwaar

Geachte heer [redacted]

Bij e-mailbericht van 20 juli 2020 heeft u een bezwaarschrift ingediend tegen mijn brief van 2 juli 2020, met kenmerk 2020.089 – 1712361-207520-WJZ in reactie op uw verzoek op grond van de Wet openbaarheid van bestuur (hierna: Wob).

Met deze brief beslis ik op uw bezwaar.

Besluit

Ik verklaar het bezwaar niet-ontvankelijk, omdat de brief van 2 juli 2020 geen besluit is. Ik heb namelijk terecht geconstateerd dat de documenten waar u om vraagt, al openbaar zijn en dat daarom de Wob niet van toepassing is. De brief heeft daardoor geen rechtsgevolg.

Ik licht mijn besluit hieronder voor u toe.

Verloop van de procedure

Bij brief van 12 mei 2020 heeft u een verzoek ingediend. U vraagt mij om u op basis van de Wob een wetenschappelijk onderzoek te doen toekomen waarin is aangetoond dat het virus SARS-CoV-2 bestaat.

Bij brief van 2 juli 2020 heb ik u laten weten dat de plicht tot openbaarmaking op grond van de Wob geen betrekking heeft op informatie die al openbaar is. De informatie waar u om vraagt is openbaar en ik heb u verwezen naar een tweetal websites.

Met uw e-mail van 20 juli 2020 heeft u bezwaar gemaakt tegen mijn brief van 2 juli 2020. Op dezelfde dag heb ik de ontvangst van uw bezwaar aan u bevestigd.

Op 23 juli 2020 heeft één van mijn medewerkers, mevrouw [redacted] een e-mail gestuurd over de bezwaarprocedure.

Beoordeling van het bezwaar

Secretaris Generaal / plv. Secretaris Generaal
Directie Wetgeving en Juridische Zaken
Cluster 1

Kenmerk
DWJZ-2020000372
1737238-209457-WJZ

Algemene toelichting

Voordat ik op uw bezwaren inga, wil ik in het algemeen iets zeggen over de regels die in dit geval gelden. Ik heb uw bezwaar beoordeeld op grond van de Algemene wet bestuursrecht (hierna: Awb) en de Wob.

Op grond van artikel 1, aanhef en onder a, van de Wob, wordt onder document verstaan een bij een bestuursorgaan berustend schriftelijk stuk of ander materiaal dat gegevens bevat.

Op grond van artikel 3, eerste lid, van de Wob kan een ieder een verzoek om informatie, neergelegd in documenten over een bestuurlijke aangelegenheid, richten tot een bestuursorgaan of een onder verantwoordelijkheid van een bestuursorgaan werkende instelling, dienst of bedrijf.

Op grond van artikel 3, tweede lid, van de Wob vermeldt de verzoeker bij zijn verzoek de bestuurlijke aangelegenheid of het daarop betrekking hebbende document, waarover hij informatie wenst te ontvangen.

Gronden van uw bezwaar

In uw bezwaarschrift heeft u aangevoerd dat ik niet heb voldaan aan uw Wob-verzoek, omdat volgens u in geen van de twee door mij genoemde wetenschappelijke publicaties sprake is van een bewijs voor het bestaan van SARS-CoV-2. Er heeft namelijk geen purificatie van een virus plaatsgevonden en er zijn geen controle-experimenten uitgevoerd. U vraagt mij de verzochte informatie alsnog toe te sturen of, als ik hier niet over beschik, dat te bevestigen. U verwijst naar de definitie van document in de Wob, waaruit volgens u niet blijkt dat een document dat al eerder is gepubliceerd, niet onder de Wob valt.

Overwegingen ten aanzien van de gronden van bezwaar

Over uw bezwaren overweeg ik het volgende.

Voordat ik inhoudelijk op uw bezwaren kan ingaan, moet ik beoordelen of uw bezwaar aan de wettelijke vereisten voldoet. Daarover overweeg ik als volgt.

Gelet op de artikelen 8:1 en 7:1, eerste lid, van de Awb kan een belanghebbende bezwaar maken tegen een besluit. Een besluit is gelet op artikel 1:3, eerste lid, van de Awb een schriftelijke beslissing van een bestuursorgaan, inhoudende een publiekrechtelijke rechtshandeling. Met het begrip rechtshandeling wordt een handeling van een bestuursorgaan bedoeld die is gericht op rechtsgevolg. Dit betekent dat door de desbetreffende handeling een wijziging in een recht of een plicht moet plaatsvinden.

Uit de rechtspraak volgt dat de plicht tot openbaarmaking op grond van de Wob geen betrekking heeft op informatie die al openbaar is.[1] In zoverre slaagt uw

[1] Onder meer de uitspraken van de Afdeling Bestuursrechtspraak van de Raad van State van 11 september 2019 (ECLI:NL:RVS:2019:3100) en 18 december 2019 (ECLI:NL:RVS:2019:4257).

Secretaris Generaal / plv. Secretaris Generaal
Directie Wetgeving en Juridische Zaken
Cluster 1

Kenmerk
DWJZ-2020000372
1737238-209457-WJZ

bezwaar dat uit de definitie van document niet blijkt dat de Wob geen betrekking heeft op openbare informatie, dus niet.

Een reactie op een verzoek om stukken openbaar te maken die al openbaar zijn, is niet op rechtsgevolg gericht en daarom geen besluit in de zin van artikel 1:3, eerste lid, van de Awb. Tegen een dergelijke mededeling kan een verzoeker, zoals u in dit geval heeft gedaan, wel bezwaar maken. In de bezwaarprocedure kan dan worden beoordeeld of het bestuursorgaan op goede gronden stelt dat de gevraagde informatie openbaar is. Ik ben van oordeel dat ik op goede gronden heb gesteld dat de door u gevraagde informatie al openbaar is. Daarom acht ik uw bezwaar niet-ontvankelijk. Dit betekent dat ik niet toekom aan een verdere inhoudelijke beoordeling van uw bezwaar. Wel licht ik hierna toe hoe ik tot de conclusie ben gekomen dat de informatie al openbaar is.

4

Naar aanleiding van uw verzoek heb ik navraag gedaan bij het RIVM. Uit deze navraag is mij gebleken dat het RIVM zich baseert op openbare informatiebronnen, waarvan de twee in mijn brief van 2 juli 2020 genoemde artikelen voorbeelden zijn. Volgens het RIVM wordt in deze twee publicaties het bestaan van het virus SARS-CoV-2 aangetoond. In mijn brief heb ik hier dan ook naar kunnen verwijzen. Er zijn overigens nog veel meer wetenschappelijke artikelen over het virus SARS-CoV-2 openbaar beschikbaar, maar op grond van de Wob ben ik niet gehouden om naar aanleiding van uw verzoek een literatuuronderzoek te doen in openbare publicaties. De Wob ziet namelijk op een bij een bestuursorgaan berustend schriftelijk stuk of ander materiaal dat gegevens bevat (artikel 1, aanhef en onder a, van de Wob). Bovendien geldt dat als vaststaat dat de gevraagde informatie al openbaar is, ook niet meer hoeft te worden nagegaan of de informatie onder het bestuursorgaan berust.[2]

Dat u van mening bent dat in de genoemde artikelen geen bewijs wordt geleverd van het bestaan van het virus, wat daar verder ook van zij, doet er niet aan af dat het RIVM onder meer uitgaat van de twee genoemde artikelen. Het valt buiten de reikwijdte van de Wob om hierover met u een (medisch) wetenschappelijke discussie te voeren.

Conclusie
Gelet op het voorgaande kom ik tot de conclusie dat uw bezwaar niet-ontvankelijk is.

Horen

Ik kan op grond van artikel 7:3, aanhef en onder c, van de Awb afzien van horen als de belanghebbende heeft verklaard geen gebruik te willen maken van het recht te worden gehoord.

[2] Uitspraak van de Afdeling Bestuursrechtspraak van de Raad van State van 18 december 2019 (ECLI:NL:RVS:2019:4257).

Secretaris Generaal / plv. Secretaris Generaal
Directie Wetgeving en Juridische Zaken
Cluster 1

Kenmerk
DWJZ-2020000372
1737238-209457-WJZ

Per e-mail van 21 juli 2020 heeft u laten weten geen gebruik te willen maken van de mogelijkheid om uw bezwaren mondeling toe te lichten. Dit betekent dat ik een beslissing neem op grond van het door u ingediende bezwaarschrift. Op grond van het bepaalde in artikel 7:3, aanhef en onder c, van de Awb zie ik af van het houden van een hoorzitting.

Hoogachtend,

de minister van Volksgezondheid,
Welzijn en Sport,
namens deze,
de secretaris-generaal,

[redacted]

U kunt tegen deze beschikking beroep instellen bij de sector bestuursrecht van de rechtbank binnen het rechtsgebied waarvan u uw woonplaats in Nederland heeft.

Het beroepschrift moet binnen zes weken na de dag waarop de beschikking u is toegezonden aan de rechtbank worden gestuurd. U kunt ook digitaal beroep instellen via http://loket.rechtspraak.nl/bestuursrecht. Daarvoor moet u wel beschikken over een elektronische handtekening (DigiD).

Het beroepschrift moet op grond van artikel 6:5 van de Algemene wet bestuursrecht zijn ondertekend en bevat ten minste de naam en adres van de indiener, de dagtekening, de omschrijving van het besluit waartegen het beroep is gericht, zo mogelijk een afschrift van dit besluit, en de gronden waarop het beroepschrift rust.

Van de indiener van het beroepschrift wordt griffierecht geheven door de griffier van de rechtbank. Nadere informatie over de hoogte van het griffierecht en de wijze van betalen wordt door de griffie van de rechtbank verstrekt.

Novet
Gary Craig Official EFT Training Center

RIVM

t.a.v. H. Burg, Directeur-generaal

Postbus 1

3720 BA Bilthoven

Amsterdam, 16 maart 2021

Betreft: Wob verzoek inzake SARS_COC-2

Geachte heer Burg,

Onder verwijzing naar bovenstaande wet, heb ik het volgende verzoek:

1. Kunt u mijn een kopie geven van alle wetenschappelijke peer-reviewed publicaties die in uw bezit zijn die de isolatie* van SARS-COV-2 virus aantonen. Het moet gaan om een monster dat rechtstreeks is afgenomen van een zieke patiënt, het monster mag niet eerst vermengd zijn met enig ander genetisch materiaal. Dit verzoek beperkt zich niet tot publicaties van het RIVM zelf, maar betreft ook enig document dat wie dan ook, waar ook ter wereld, heeft gepubliceerd over de isolatie van SARS-COV-2 en dat in uw bezit is.

   *Isolatie wordt hier gebruikt in de letterlijke betekenis, dus het scheiden van een iets van iets anders. Ik vraag niet om een publicaties die het hebben over cultiveren of PCR gebruiken voor het vermeerderen van genetische materiaal noch publicaties die iets 'sequencing' obv computermodellen.

2. Kunt u mij een kopie geven van alle wetenschappelijk publicaties die in uw bezit zijn die bewijzen dat SARS-COV-2 de veroorzaker is van de ziekte 'COVID-19' conform het Koch postulaat, waarbij aan alle 4 de gestelde voorwaarden is voldaan?

Ik ontvang alle informatie graag in pdf formaat via mail.

Ik zie uw reactie met belangstelling tegemoet.

Hartelijke groet,

Gabriëlle Rutten
Directeur Novet – het Gary Craig Official EFT Training Center

Novet
Gary Craig Official EFT Training Center

RIVM
Attn: H. Burg, Director-General
P.O. Box 1
3720 BA Bilthoven

Amsterdam, 16 March 2021

Subject: Wob request regarding SARS_COC-2

Dear Mr Burg,

With reference to the above law, I have the following request:

1. Could you please provide mE with a copy of all scientific peer-reviewed publications in your possession that demonstrate the isolation* of SARS-COV-2 virus. It must be a sample taken directly from a sick patient, the sample must not have been first mixed with any other genetic material. This request is not limited to publications of the RIVM itself, but also concerns any document published by anyone, anywhere in the world, regarding the isolation of SARS-COV-2 that is in your possession.
*Isolation is used here in the literal sense, i.e. separating one thing from another. I am not asking for any publications that talk about cultivating or using PCR to propagate genetic material nor publications that 'sequence' anything obv computer models.

2. Can you give me a copy of all scientific publications in your possession that prove that SARS-COV-2 is the causative agent of the disease 'COVID-19' according to the Koch postulate, where all 4 conditions are met?

I would like to receive all information in pdf format via mail.

I look forward to your response with interest.

Kind regards,
Gabrielle Rutten Director Novet - the Gary Craig Official EFT Training Center

Gabriëlle Rutten
Directeur Novet – het Gary Craig Official EFT Training Center

Translated with www.DeepL.com/Translator (free version)

Ministerie van Volksgezondheid, Welzijn en Sport

> Retouradres Postbus 20350 2500 EJ Den Haag

UITSLUITEND PER E-MAIL
De heer G. Rutten
info@official-eft.nl

Directie Wetgeving en Juridische Zaken
Bezoekadres:
Parnassusplein 5
2511 VX Den Haag
T 070 340 79 11
F 070 340 59 84
www.rijksoverheid.nl

Inlichtingen bij
mr. B. Gomes Caixinha Knaff
Cluster Wob
vws.wob@minvws.nl
T 070 340 5564
T (b.g.g.) 070 340 5400

Ons kenmerk
2021.063
2350619-1007570-WJZ

Uw e-mail van
16 maart 2020

Bijlage

Correspondentie uitsluitend richten aan het retouradres met vermelding van de datum en het kenmerk van deze brief.

Datum 26 april 2021
Betreft Verzoek om informatie

Geachte heer Rutten,

Op 16 maart 2021 ontving ik uw e-mail. De ontvangst hiervan heb ik bevestigd bij brief van 29 maart 2021 met kenmerk 2021.063/001.

Met een beroep op de Wet openbaarheid van bestuur (hierna: Wob) verzoekt u om alle wetenschappelijke peer-reviewed publicaties die de isolatie van het SARS-COV-2 aantonen. Verder heeft u verzocht om wetenschappelijke publicaties die bewijzen dat voornoemd virus de veroorzaker is van de ziekte 'Covid-19'.

De Wob is overeenkomstig artikel 6, eerste lid enkel van toepassing op informatie die is neergelegd in documenten die niet reeds openbaar zijn. De door u gevraagde informatie betreft openbare informatie. Hiernavolgend zal ik aangeven waar u de betreffende informatie kunt vinden.

Het coronavirus Sars-Cov-2 is het virus dat de ziekte COVID-19 kan veroorzaken. Meer informatie hierover kunt u vinden op de website van het Rijkinstituut voor Volksgezondheid en Milieu (hierna: RIVM) onder het kopje 'virus' (www.rivm.nl/coronavirus-covid-19/virus). Voor de wetenschappelijke rapporten over het bestaan van Sars-Cov2 verwijs ik u naar de volgende websites: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7159086/ en https://pubmed.ncbi.nlm.nih.gov/31978945/.

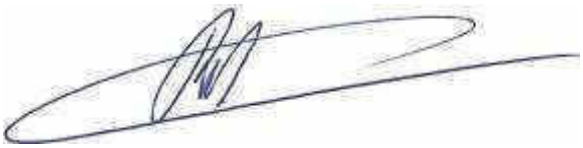
Naast de hierboven genoemde website van het RIVM, wil ik ook op de volgende website van het RIVM wijzen: https://lci.rivm.nl/richtlijnen/covid-19. Op deze website treft u relevante literatuur aan.

Mocht u nog vragen hebben over deze brief, neemt u dan gerust contact op met

het Wob-cluster. De contactgegevens staan bovenaan deze brief.

Hoogachtend,

de Minister van Volksgezondheid,
Welzijn en Sport,
namens deze,
de directeur Wetgeving en Juridische Zaken,



mr. M.M. den Boer

Directie Wetgeving en Juridische Zaken

Ons kenmerk
2021.063
2350619-1007570-WJZ

(English Translation of response from the Dutch)
Ministry of Health, Well-being and Sport

> Return address PO Box 20350 2500 EJ The Hague

BY E-MAIL ONLY
Mr G. Rutten
info@official-eft.nl

Date 26 april 2021
Subject Request for information

Dear Mr Rutten,

On March 16, 2021, I received your email. I confirmed receipt of this letter of 29 March 2021 with characteristic 2021.063/001.

By invoking the Public Administration Act (hereinafter: Wob), you request all scientific peer-reviewed publications that promote the isolation of the SARSCOV 2. You have also requested scientific publications that prove that the aforementioned virus is the cause of the disease 'Covid-19'.

In accordance with Article 6(1), the Wob only applies to information documented in documents which are not already public. The requested information concerns public information. Next I will indicate where you can find the relevant information.

The coronavirus Sars-Cov-2 is the virus that can cause the disease COVID-19. More information about this can be found on the website of the Government Institute for Public Health and the Environment (hereinafter: RIVM) under the heading 'virus' (www.rivm.nl/coronavirus-covid-19/virus). For the scientific reports about the existence of Sars-Cov2 I refer you to the following websites:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7159086/ and
https://pubmed.ncbi.nlm.nih.gov/31978945/.

In addition to the website of the RIVM mentioned above, you might also like to visit the following RIVM website: https://lci.rivm.nl/richtlijnen/covid-19. On this website you will find relevant literature.

If you have any questions about this letter, please feel free to contact the Wob cluster. The contact details are at the top of this letter.

Sincerely,

the Minister for Health,
Well-being and Sport,
on behalf of this,
the Director of Legislation and Legal Affairs,
Mr. M.M. den Boer

Offentleglova: Royal Norwegian Ministry of Health and Care Services

Inbox

Martiens Bekker 28/12/2020

to Jon

From Martiens Bekker • artrikascrapart@gmail.com

To [redacted] • [redacted]@vinje.kommune.no

Date 28 Dec 2020 15:19

See security details

[redacted]

I wish to apply for the following information from the Royal Norwegian Ministry of Health and Care Services and thought it appropriate to do so through my immediate authorities.

Requesting a full accurate and complete list of records held by or under the authority of the Royal Norwegian Ministry of Health and Care Services, which describes

I wish to apply for the following information from the Royal Norwegian Ministry of Health and Care Services and thought it appropriate to do so through my immediate authorities.

Requesting a full accurate and complete list of records held by or under the authority of the Royal Norwegian Ministry of Health and Care Services, which describes the isolation of the SARS-COV-2 virus causing the Covid19 decease, taken directly from a symptomatic patient with Covid 19 where the sample was not combined or mixed with any other source of genetic material, thereby eliminating contamination as a possible alternative source of sampling.

I am at this point motivated to do so due to the many international concerns arising concerning the use of vaccines. I am also concerned about the level and standards of scientific based regulations mandated and or recommended by the Norwegian Authorities and authorities abroad.

My reason to do so is based on the fact that I have been officially diagnosed with Narcolepsy, in Oslo 2017.
Although this confirmed that I have had this condition since 1983, I assumed the symptoms I suffered was from an accident in that same year. Based on my assumption that Narcolepsy symptoms started, after I suffered head trauma in an accident, the Neurologist

assumption that Narcolepsy symptoms started, after I suffered head trauma in an accident, the Neurologist in Skien had a full MRI brain scan done. The result was that I have no significant brain damage that could have led to developing Narcolepsy.

I was unofficially diagnosed in 1991 and although I did not want to accept it, I have studied the condition now for a number of years.
Since I was officially diagnosed I have been looking into the research done in Norway, on the **2009 Pandemrix Influenza Vaccination** that have caused a significant increase Narcolepsy.
Based on the excellent research by the Norwegian Health Services, I came to the realisation that my Narcolepsy had most likely been caused by a vaccine I received in 1983. At that time I was a 16yr old South African and we were given a vaccine to prepare us for when we will begin with conscript military service at an age of 17 years.
At the moment I am in the process of determining what type of vaccine I received in 1983, but getting information related to the 'Apartheid' National Party Government is difficult.

Sound scientific evidence in the **Pandemrix** case, shows that the vaccine cause an Autoimmune response, resulting in the immune system killing off the neurons located predominantly in the perifornical area and lateral hypothalamus, responsible for the

Sound scientific evidence in the **Pandemrix** case, shows that the vaccine cause an Autoimmune response, resulting in the immune system killing off the neurons located predominantly in the perifornical area and lateral hypothalamus, responsible for the production of the neurotransmitter called Hypocretin. The **2009 Pandemrix Influenza Vaccination** and most likely other related vaccines, have caused irreparable humiliating suffering for many, due to watered down science and profiteering.

Narcolepsy has effected every aspect of my life for the last 34 years and I would not wish it upon any one.

None of the above makes me a qualified medical doctor or scientist (neither does being a billionaire make Bill Gates a qualified medical doctor or scientist), but I am however an expert in living with Narcolepsy and my concerns are more than justified!

I believe that all humans have the right to make informed decisions and at the moment I have failed to find any substantial evidence. Therefore I hope the Royal Norwegian Ministry of Health and Care Services will be able to do so.

I realise my request comes at a difficult time and will probably not be received positively, with further possible negative actions taken against me in person.

The **2009 Pandemrix influenza vaccination** and most likely other related vaccines, have caused irreparable humiliating suffering for many, due to watered down science and profiteering.

Narcolepsy has effected every aspect of my life for the last 34 years and I would not wish it upon any one.

None of the above makes me a qualified medical doctor or scientist (neither does being a billionaire make Bill Gates a qualified medical doctor or scientist), but I am however an expert in living with Narcolepsy and my concerns are more than justified!

I believe that all humans have the right to make informed decisions and at the moment I have failed to find any substantial evidence. Therefore I hope the Royal Norwegian Ministry of Health and Care Services will be able to do so.

I realise my request comes at a difficult time and will probably not be received positively, with further possible negative actions taken against me in person.

Helsing og god nytt år til derre

Fra: Riise Øystein [mailto:Oystein.Riise@hod.dep.no]
Sendt: onsdag 13. januar 2021 14:37
Til: [redacted]@vinje.kommune.no>
Emne: krav om innsyn

Hei,

Vi viser til din henvendelse om krav om innsyn fra en innbygger i din kommune.

Slik vi forstår henvendelsen gjelder det dokumentasjon på om SARS-CoV-2 forårsaker sykdommen covid-19.

Departementet vil vise til Folkehelseinstituttets hjemmesider på norsk og engelsk Folkehelseinstituttet - FHI :

Det finnes også informasjon på nettsidene til det europeiske smittevernbyrået Homepage | European Centre for Disease Prevention and Control (europa.eu) og WHO Technical guidance (who.int).

Med vennlig hilsen

Helse- og

omsorgsdepartementet

Øystein Riise
Spesialrådgiver
Folkehelseavdelingen

Mobil: 97668432

English Translation

Hi,

We refer to your request for access from a resident of your municipality.

As we understand the inquiry, there is documentation of whether SARS-CoV-2 causes the disease covid-19.

The Ministry will refer to the National Institute of Public Health's websites in Norwegian and English
Folkehelseinstituttet - FHI :

Information is also available on the website of the European Agency for Communicable Disease Control

Homepage | European Centre for Disease Prevention and Control (europa.eu) and WHO Technical guidance (who.int).

Attachments area

Subject **Information request (ref: 2161889)**
From Courtenay <Courtenay@manx.net>
To Christine Massey <christinem@fluoridefreepeel.ca>
Date 2022-01-19 04:58 AM

roundcube

- Response (not held).pdf (~167 KB)

FYI.

From: Department of Health and Social Care <dhsc@foi.gov.im>
Date: 19 January 2022 at 09:09:19 GMT
To: courtenay@manx.net
Subject: Information request (ref: 2161889)

Information request
Our reference: 2161889

Dear Mr Adam-lawrence

Thank you for your request for information received on 23 December 2021.

Please find attached our response to your request.

Rebecca Evans
Corporate Services
695794
rebecca.evans2@gov.im

Isle of Man Government
Reiltys Ellan Vannin

Department of Health and Social Care

Rheynn Slaynt as Kiarail y Theay

Mr Courtenay Adam-Lawrence
Richmond House
Richmond Road
Isle of Man
IM8 3PB

Interim Chief Executive: Karen Malone
Freedom of Information Team
First Floor
Belgravia House
Douglas
Isle of Man
IM1 1AE

Our ref: 2161889

19 January 2022

Dear Mr Adam-Lawrence

We write further to your request which was received on 23 December 2021 and which states:

"1) Please provide a whole genomic identification of the omicron strain of the SARS-CoV-2 virus, not a partial sequence

2) Please confirm test procedures to identify an omicron virus and the method statement of how it is purified and isolated

3) Please provide all evidence of omicron contagion in men and women

4) Please confirm that the omicron strain has a unique sequence and was obtained from saliva, mucus, blood or urine from a man, woman or animal.

5) Please confirm that omicron is not an in silico computer made up model of a strain of a virus

thank you,"

Our response:

While our aim is to provide information whenever possible, in this instance the Department of Health and Social Care ('the Department') is unable to provide the information that you have requested. This is in line with Section 11(3)a of the Act, as a practical refusal reason applies; namely we do not hold or cannot, after taking reasonable steps to do so, find the information that you have requested as it may be held by **Manx Care**.

You may wish to re-submit your request to **Manx Care** which is an option available on the Freedom of Information request portal, who may be able to help you. To provide advice and assistance, the Department of Health and Social Care redesigned on 1 April 2021 as a direct result of Sir Jonathan Michael's Independent Review of the Isle of Manx Health and Care System. This Review continues to be a catalyst for change and improved service provision.

The redesigned Department ensures the separation between the setting of policy and strategy and the delivery of services by Manx Care.

Please quote the reference number 2161889 in any future communications.

Your right to request a review

If you are unhappy with this response to your freedom of information request, you may ask us to carry out an internal review of the response, by completing a complaint form and submitting it electronically or by delivery/post.

An electronic version of our complaint form can be found by going to our website at https://services.gov.im/freedom-of-information/Review . If you would like a paper version of our complaint form to be sent to you by post, please contact me and I will be happy to arrange for this. Your review request should explain why you are dissatisfied with this response, and should be made as soon as practicable. We will respond as soon as the review has been concluded.

If you are not satisfied with the result of the review, you then have the right to appeal to the Information Commissioner for a decision on;

1. Whether we have responded to your request for information in accordance with Part 2 of the Freedom of Information Act 2015; or
2. Whether we are justified in refusing to give you the information requested.

In response to an application for review, the Information Commissioner may, at any time, attempt to resolve a matter by negotiation, conciliation, mediation or another form of alternative dispute resolution and will have regard to any outcome of this in making any subsequent decision.

More detailed information on your right to a review can be found on the Information Commissioner's website at www.inforights.im.

Should you have any queries concerning this letter, please do not hesitate to contact me.

Further information about freedom of information requests can be found at www.gov.im/foi.

I will now close your request as of this date.

Yours sincerely

Rebecca Evans

Subject **Information request (ref: 2161990)**
From Courtenay <Courtenay@manx.net>
To Christine Massey <christinem@fluoridefreepeel.ca>
Date 2022-01-19 04:59 AM

roundcube

- Response (not held).pdf (~167 KB)

FYI.

From: Department of Health and Social Care <dhsc@foi.gov.im>
Date: 19 January 2022 at 09:20:29 GMT
To: courtenay@manx.net
Subject: Information request (ref: 2161990)

Information request
Our reference: 2161990

Dear Mr Adam-lawrence

Thank you for your request for information received on 23 December 2021.

Please find attached our response to your request.

Rebecca Evans
Corporate Services
695794
rebecca.evans2@gov.im

Isle of Man Government
Reiltys Ellan Vannin

Department of Health and Social Care

Rheynn Slaynt as Kiarail y Theay

Mr Courtenay Adam-Lawrence
Richmond House
Richmond Road
Isle of Man
IM8 3PB

Interim Chief Executive: Karen Malone
Freedom of Information Team
First Floor
Belgravia House
Douglas
Isle of Man
IM1 1AE

Our ref: 2161990

19 January 2022

Dear Mr Adam-Lawrence

We write further to your request which was received on 23 December 2021 and which states:

"1) Please provide a whole genomic identification of the kent strain of the SARS-CoV-2 virus, not a partial sequence

2) Please confirm test procedures to identify a kent strain virus and the method statement of how it is purified and isolated

3) Please provide all evidence of kent contagion in men and women

4) Please confirm that the kent strain has a unique sequence and was obtained from saliva, mucus, blood or urine from a man, woman or animal.

5) Please confirm that kent is not an in silico computer made up model of a strain of a virus

thank you,"

Our response:

While our aim is to provide information whenever possible, in this instance the Department of Health and Social Care ('the Department') is unable to provide the information that you have requested. This is in line with Section 11(3)a of the Act, as a practical refusal reason applies; namely we do not hold or cannot, after taking reasonable steps to do so, find the information that you have requested as it may be held by **Manx Care**.

You may wish to re-submit your request to **Manx Care** which is an option available on the Freedom of Information request portal, who may be able to help you. To provide advice and assistance, the Department of Health and Social Care redesigned on 1 April 2021 as a direct result of Sir Jonathan Michael's Independent Review of the Isle of Manx Health and Care System. This Review continues to be a catalyst for change and improved service provision.

The redesigned Department ensures the separation between the setting of policy and strategy and the delivery of services by Manx Care.

Please quote the reference number 2161990 in any future communications.

Your right to request a review

If you are unhappy with this response to your freedom of information request, you may ask us to carry out an internal review of the response, by completing a complaint form and submitting it electronically or by delivery/post.

An electronic version of our complaint form can be found by going to our website at https://services.gov.im/freedom-of-information/Review . If you would like a paper version of our complaint form to be sent to you by post, please contact me and I will be happy to arrange for this. Your review request should explain why you are dissatisfied with this response, and should be made as soon as practicable. We will respond as soon as the review has been concluded.

If you are not satisfied with the result of the review, you then have the right to appeal to the Information Commissioner for a decision on;

1. Whether we have responded to your request for information in accordance with Part 2 of the Freedom of Information Act 2015; or
2. Whether we are justified in refusing to give you the information requested.

In response to an application for review, the Information Commissioner may, at any time, attempt to resolve a matter by negotiation, conciliation, mediation or another form of alternative dispute resolution and will have regard to any outcome of this in making any subsequent decision.

More detailed information on your right to a review can be found on the Information Commissioner's website at www.inforights.im.

Should you have any queries concerning this letter, please do not hesitate to contact me.

Further information about freedom of information requests can be found at www.gov.im/foi.

I will now close your request as of this date.

Yours sincerely

Rebecca Evans

Gem For Your Collection

[redacted] Wed, Feb 2, 2022 at 3:04 AM
Reply-To: [redacted]
To: Christine Massey <cmssyc@gmail.com>, christinem <christinem@fluoridefreepeel.ca>, "christine.massey" <christine.massey@protonmail.com>

Dear Christine,

We received another response (this time honest!) from ICMR. When pushed to the wall, they responded thus:

No published peer-reviewed publication, that describes direct purification of SARS-CoV-2 from patient samples, could be found after extensive literature research. Various papers, like the one, shared earlier, have followed a similar methodology of inoculation of patient samples in cell culture & then the characterization of the virus. This is the best paper that can be provided at this point in time.

Please find attached the full FOI (we had to appeal after 1 standard response).

Thanks

Not_Found_After_Extensive_Search_VirusIsolationINCMRJan2022ST.pdf
781K

Online RTI Request Form Details

RTI Request Details :-

RTI Request Registration number	INCMR/R/T/21/01307
Public Authority	Indian Council of Medical Research

Personal Details of RTI Applicant:-

Name	[redacted]
Gender	[redacted]
Address	[redacted]
Country	India
State	[redacted]
Status	Details not provided
Educational Status	Details not provided
Phone Number	Details not provided
Mobile Number	Details not provided
Email-ID	[redacted]

Request Details :-

Citizenship	Indian
Is the Requester Below Poverty Line ?	[redacted]
BPL Card No. *(Proof of BPL may be provided as an attachment)*	Details not provided
Year of Issue	Details not provided
Issuing Authority	Details not provided

(Description of Information sought (upto 500 characters)

Description of Information Sought	
attached	
Concerned CPIO	Dr Nivedita Gupta
Supporting document *(only pdf upto 1 MB)*	

Print Close

Please provide all studies and/or reports in the possession, custody or control of ICMR or NIV or other affiliated bodies/agencies describing the purification of the alleged "COVID-19 virus" (aka "SARS-COV-2", including any alleged "variants") directly from a sample taken from a diseased human, where the patient sample was not first combined with any other source of genetic material (i.e. monkey kidney cells aka Vero cells; fetal bovine serum).

Clarification of Request:

Please note that I am NOT requesting studies/reports where researchers failed to purify the suspected "virus" and instead:

1. cultured something, and/or
2. performed an amplification test (i.e. PCR), and/or
3. fabricated a genome from sequences detected in an impure substance, and/or
4. produced electron microscopy images of unpurified things.

I am already aware that according to virus theory a "virus" requires host cells in order to replicate, and am not requesting records that describe replication of a 'virus' without host cells. Nor am I requesting records that describe a strict fulfillment of Koch's Postulates (or Rivers's criteria), or records that describe a suspected "virus" floating in a vacuum, or private patient information.

I am simply requesting records that describe purification (separation of the alleged virus from everything else in the patient sample, as per standard laboratory practices for the purification of other very small things). This would normally involve maceration, filtration, and ultra-centrifugation.

Please note that my request includes any study/report matching the above description, authored by anyone, anywhere.

If any records match the above description of requested records and are currently available in the public domain, please provide enough information about each record so that I may identify and access each one with certainty (i.e. title, author(s), date, journal, where the public may access it). Please provide URLs where possible.

Please do not point me to or send me papers such as the following:

1 Abraham Priya, Cherian Sarah, Potdar Varsha. Genetic characterization of SARS-CoV-2 & implications for epidemiology, diagnostics & vaccines in India. 2020,152 (1), 12-15.

2 Sarkale P, Patil S, Yadav PD, et al. First isolation of SARS-CoV-2 from clinical samples in India. Indian J Med Res. 2020;151(2 & 3):244-250. doi:10.4103/ijmr.IJMR_1029_20.

These are not what I am looking for.

Select Language: English

Public Authorities Available

RTI Online

Version 2.0

An Initiative of Department of Personnel & Training, Government of India

Online RTI Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	INCMR/R/T/21/01307
Name	[redacted]
Received Date	08/11/2021
Public Authority	Indian Council of Medical Research
Status	REQUEST DISPOSED OF
Date of action	03/12/2021
Reply :- please find attached pdf	
View Document	
CPIO Details :-	Dr Nivedita Gupta Phone: 011-26588980 ngupta[at]icmr[dot]org[dot]in
First Appellate Authority Details :-	Dr Samiran Panda1 Phone: 011-26588272 samiranpanda[dot]hq[at]icmr[dot]gov[dot]in
Nodal Officer Details :-	
Telephone Number	011-26588980
Email Id	maheshchand[dot]hq[at]icmr[dot]gov[dot]in

Print RTI Application Print Status Go Back

Kindly refer to this article that describes virus isolation, sequencing and electron microscopy from original Wuhan patients of SARS-CoV-2
https://www.nejm.org/doi/10.1056/NEJMoa2001017?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0www.ncbi.nlm.nih.gov

Online RTI Appeal Form Details

RTI Appeal Details :-

RTI Appeal Registration number	INCMR/A/E/21/00228
Public Authority	Indian Council of Medical Research

Personal Details of Appellant:-

Request Registration Number	INCMR/R/T/21/01307
Request Registration Date	08/11/2021
Name	[redacted]
Gender	[redacted]
Address	[redacted]
Country	India
State	[redacted]
Status	Details not provided
Educational Status	Details not provided
Phone Number	Details not provided
Mobile Number	Details not provided
Email-ID	[redacted]

Appeal Details :-

Citizenship	Indian
Is the Requester Below Poverty Line ?	Yes
Ground For Appeal	Provided Incomplete,Misleading or False Information
CPIO of Public Authority approached	Dr Nivedita Gupta
CPIO's Order/Decision Number	Details not provided
CPIO's Order/Decision Date	

(Description of Information sought (upto 500 characters)

Prayer or Relief Sought
I have clearly mentioned that I am not looking for papers such as the one provided by you. I am requesting records that describe purification (separation of the alleged virus from everything else in the patient sample, as per standard laboratory practices for the purification of other very small things). This would normally involve maceration, filtration, and ultra-centrifugation. Please note that my request includes any study/report matching the above description, authored by anyone, anywhere in the world

Supporting document ***(only pdf upto 1 MB)***	Supporting document not provided

Print Close

Select Language: English

Public Authorities Available

RTI Online

Version 2.0

An Initiative of Department of Personnel & Training, Government of India

Home Submit Request Submit First Appeal View Status View History new Login User Manual Contact Us FAQ

Online RTI First Appeal Status Form

Note:Fields marked with * are Mandatory.

Enter Registration Number	INCMR/A/E/21/00228
Name	[redacted]
Received Date	18/12/2021
Public Authority	Indian Council of Medical Research
Status	APPEAL DISPOSED OF
Date of action	31/01/2022
Reply :- No published peer-reviewed publication, that describes direct purification of SARS-CoV-2 from patient samples, could be found after extensive literature research. Various papers, like the one, shared earlier, have followed a similar methodology of inoculation of patient samples in cell culture & then the characterization of the virus. This is the best paper that can be provided at this point in time.	
First Appellate Authority Details :-	Dr Samiran Panda1 Phone: 011-26588272 samiranpanda[dot]hq[at]icmr[dot]gov[dot]in
Nodal Officer Details :-	
Telephone Number	011-26588980
Email Id	maheshchand[dot]hq[at]icmr[dot]gov[dot]in

Print RTI First Appeal Print Status Go Back

Final Status of INCMR/R/E/21/01028

Applicant Name	[redacted]
Date of receipt	31/01/2022
Request Filed With	Indian Council of Medical Research
Text of Application	IS THERE ANY PHYSICAL SAMPLE OF PURIFIED SARS COV 2 VIRUS EXISTS BEFORE YOU. I MEAN PURIFIED VIA DENSITY GRADIENT CENTRIFUGATION. IF SO THEN PLEASE PUBLISH THE ARTICLE EXPLAINING THE SARS COV 2 VIRUS PURIFICATION
Request document *(if any)*	document not provided
Status	**REQUEST DISPOSED OF** as on 14/02/2022
Date of Action	14/02/2022
Remarks	**Reply** :- Such a sample is not available with ICMR.

Print

From [redacted]
To <christinem@fluoridefreepeel.ca>
Date 2022-03-24 04:13 AM

roundcube

Hi Christine
I sent Freedom of information request to finnish health authority (THL) and go the (expected) answer. You can see them below.
[redacted] from Finland

Their response:
Hey,
We have examined your request for information on 16.3.2022.
The right of access to information pursuant to Chapter 3 of the Act on Public Access to the Activities of Public Authorities (621/1999, hereinafter "the Public Access to Information Act") applies to information entered in the documents of public authorities. According to section 5 (3) (3) of the Public Access to Information Act, documents obtained for the authority's internal training, information retrieval or other comparable internal use are not considered to be documents of the authority. For example, scientific literature and libraries collected by the authority for its own activities are therefore not documents of the authority that would be provided in response to requests for information under the Public Access to Information Act. As the right of access under Chapter 3 of the Public Access to Information Act is only information contained in existing documents, new documents (such as bibliographies) are in principle produced in response to requests.
Link to the Publicity Act: https://www.finlex.fi/fi/laki/ajantasa/1999/19990621#L1P5

THL has examined whether it is in possession of authority documents, the subject of your request for a reply. No such documents have been found. Given that the term 'isolate' has been used in a different sense in the request for information than is well established in science and that 'isolating' any virus within the meaning of the request for information is not at all possible, it is likely that the entire file does not exist at all.

If you wish, you have the right to have the matter confirmed by an authority. If you would like the matter to be referred to an authority for confirmation, you can report it by replying to this message.

Regards,
Tuuli Karppinen
Lawyer
Department of Health and Welfare

My request sent to finnish Department of Health and Welfare (THL) is as you recommended it to be done:

Aihe: Tietojen saantipyyntö: SARS-COV-2:n ja sen varianttien eristämiseen liittyvät lähteet

Marjo Loisa
Terveyden ja hyvinvoinnin laitos
Viestintä ja vaikuttaminen -yksikkö
Viestintäjohtaja
PL 30
00271 Helsinki
Lähetetty sähköpostitse osoitteeseen: marjo.loisa@thl.fi

Tämä on julkisuuslain nojalla tehty virallinen pyyntö.
https://oikeusministerio.fi/esite-julkisuuslaista
https://www.finlex.fi/fi/laki/ajantasa/1999/19990621#L4

Kuvaus pyydetyistä lähteistä:
Kaikki Terveyden ja hyvinvoinnin laitoksen (THL) hallussa tai tiedossa olevat lähteet, jotka kuvaavat sars-cov-2-viruksen eristämistä (mukaan lukien kaikki oletetut variantit) suoraan sairaalta potilaalta otetusta näytteestä, jossa potilasnäytettä ei ole ensin yhdistetty muuhun geneettisen materiaalin lähteeseen (esim. apinan munuaissoluihin, maksasyöpäsoluihin, naudan sikiön seerumiin jne).

Pyydän huomioimaan, että käytän "eristämistä" sanan arkipäiväisessä merkityksessä, eli asian (asioiden) -tässä tapauksessa sars-covid-2 viruksen (tai sen varianttien) erottamiseen kaikesta muusta. En siis pyydä lähteitä, joissa "sars-cov-2:n eristämisellä" viitataan:

* viljelyyn tai
* monistukseen (eli PCR-testiin) tai
* sekvensointiin tai
* elektronimikroskooppikuviin puhdistamattomasta näytteestä.

Pyydän siis lähteitä, joissa käy ilmi viruksen eristäminen, eli jossa oletettu virus on erotettu kaikesta muusta suoraan potilaalta otetusta näytteestä tavallisten laboratoriokäytäntöjen mukaisesti, joilla eristetään hyvin pieniä asioita.

Pyyntöni sisältää kaikki yllä olevaa kuvausta vastaavat tutkimukset/raportit, jotka kuka tahansa on tuottanut missä päin maailmaa tahansa.
Pyyntöni ei siis rajoitu lähteisiin, jotka THL on kirjoittanut tai toimittanut tai jotka liittyvät THL:n tekemään työhön. Pyyntöni sisältää kaikki lähteet, esimerkiksi (mutta ei rajoittuen) kaikki julkaistut vertaisarvioidut tutkimukset, jotka THL on ladannut tai tulostanut ja jotka ovat THL:n tiedossa ja päätösten perustalla.

Jos saatavillanne ja käytössänne olevista lähteistä jokin vastaa yllä olevaa kuvausta ja on tällä hetkellä julkisesti saatavilla muualla, pyydän jokaisesta lähteestä riittävästi tietoa, jotta voin varmuudella löytää sen (esim. nimi, tekijä(t), päivämäärä, lehti, johon on julkinen pääsy). Pyydän myös URL-osoitteita mahdollisuuksien mukaan.
Pyydän dokumentteja pdf-muodossa sähköpostiin.

Yhteystiedot:

Sukunimi: [redacted]

Etunimi: [redacted]

Osoite: [redacted]

Puhelinnumero: [redacted]

Sähköposti:
[redacted]

Kiitos jo etukäteen!

Subject **Fwd: Vastaus. Tietojen saantipyyntö: SARS-COV-2:n ja sen varianttien eristämiseen liittyvät lähteet**

From [redacted]

To <christinem@fluoridefreepeel.ca>

Date 2022-03-30 02:22 AM

Hi Christine

Here is the original email I got from finnish health authority (THL) and the Freedom of information request I sent to them.

I wish my name and contact information would not be visible

Blessings:[redacted]

---------- Forwarded message ---------
Lähettäjä: **THL Kirjaamo** <kirjaamo@thl.fi>
Date: pe 18. maalisk. 2022 klo 14.21
Subject: Vastaus. Tietojen saantipyyntö: SARS-COV-2:n ja sen varianttien eristämiseen liittyvät lähteet
To:[redacted]

Hei,

Olemme tutkineet 16.3.2022 tekemänne tietopyynnön.

Lain viranomaisen toiminnan julkisuudesta (621/1999, jäljempänä "julkisuuslaki") 3 luvun mukaiset tiedonsaantioikeudet kohdistuvat *viranomaisen asiakirjoihin* merkittyihin tietoihin. Julkisuuslain 5 § 3 momentin 3-kohdan mukaan viranomaisen asiakirjoina ei pidetä viranomaisen sisäistä koulutusta, tiedonhakua tai muuta niihin verrattavaa sisäistä käyttöä varten hankittuja asiakirjoja. Esimerkiksi viranomaisen omaa toimintaansa varten keräämä tieteellinen kirjallisuus ja kirjastot eivät siten ole viranomaisen asiakirjoja, joita toimitettaisiin vastauksena julkisuuslain mukaisiin tietopyyntöihin. Koska julkisuuslain 3-luvun mukaiset tiedonsaantioikeudet koskevat vain jo olemassa oleviin asiakirjoihin sisältyviä tietoja, pyyntöihin vastattaessa ei lähtökohtaisesti tuoteta uusia asiakirjoja (kuten kirjallisuusluetteloita).

Linkki julkisuuslakiin: https://www.finlex.fi/fi/laki/ajantasa/1999/19990621#L1P5

THL on tutkinut onko sen hallussa viranomaisen asiakirjoja, joita pyyntönne koskee. Tällaisia asiakirjoja ei ole löytynyt. Huomioiden se, että tietopyynnössä käsitettä "eristää" on käytetty eri merkityksessä kuin tieteessä vakiintuneesti ja minkään viruksen "eristäminen" tietopyynnössä tarkoitetulla tavalla ei liene ylipäätään mahdollista, on luultavaa, että tällaisia asiakirjoja ei ylipäätään ole olemassa.

Teillä on halutessanne oikeus saada asia viranomaisen ratkaistavaksi. Mikäli haluatte, että asia siirretään viranomaisen ratkaistavaksi, voitte ilmoittaa siitä vastaamalla tähän viestiin.

Ystävällisin terveisin,

Tuuli Karppinen

Lakimies

Terveyden ja hyvinvoinnin laitos

Lähettäjä [redacted]
Lähetetty: [redacted]
Vastaanottaja: Loisa Marjo (THL) <marjo.loisa@thl.fi>
Aihe: Tietojen saantipyyntö: SARS-COV-2:n ja sen varianttien eristämiseen liittyvät lähteet

Aihe: Tietojen saantipyyntö: SARS-COV-2:n ja sen varianttien eristämiseen liittyvät lähteet

Marjo Loisa
Terveyden ja hyvinvoinnin laitos
Viestintä ja vaikuttaminen -yksikkö
Viestintäjohtaja
PL 30
00271 Helsinki
Lähetetty sähköpostitse osoitteeseen: marjo.loisa@thl.fi

Tämä on julkisuuslain nojalla tehty virallinen pyyntö.
https://oikeusministerio.fi/esite-julkisuuslaista
https://www.finlex.fi/fi/laki/ajantasa/1999/19990621#L4

Kuvaus pyydetyistä lähteistä:
Kaikki Terveyden ja hyvinvoinnin laitoksen (THL) hallussa tai tiedossa olevat lähteet, jotka kuvaavat sars-cov-2-viruksen eristämistä (mukaan lukien kaikki oletetut variantit) suoraan sairaalta potilaalta otetusta näytteestä, jossa potilasnäytettä ei ole ensin yhdistetty muuhun geneettisen materiaalin lähteeseen (esim. apinan munuaissoluihin, maksasyöpäsoluihin, naudan sikiön seerumiin jne).

Pyydän huomioimaan, että käytän "eristämistä" sanan arkipäiväisessä merkityksessä, eli asian (asioiden) -tässä tapauksessa sars-covid-2 viruksen (tai sen varianttien) erottamiseen kaikesta muusta. En siis pyydä lähteitä, joissa "sars-cov-2:n eristämisellä" viitataan:
* viljelyyn tai
* monistukseen (eli PCR-testiin) tai
* sekvensointiin tai
* elektronimikroskooppikuviin puhdistamattomasta näytteestä.

Pyydän siis lähteitä, joissa käy ilmi viruksen eristäminen, eli jossa oletettu virus on erotettu kaikesta muusta suoraan potilaalta otetusta näytteestä tavallisten laboratoriokäytäntöjen mukaisesti, joilla eristetään hyvin pieniä asioita.

Pyyntöni sisältää kaikki yllä olevaa kuvausta vastaavat tutkimukset/raportit, jotka kuka tahansa on tuottanut missä päin maailmaa tahansa.
Pyyntöni ei siis rajoitu lähteisiin, jotka THL on kirjoittanut tai toimittanut tai jotka liittyvät THL:n tekemään työhön. Pyyntöni sisältää kaikki lähteet, esimerkiksi (mutta ei rajoittuen) kaikki julkaistut vertaisarvioidut tutkimukset, jotka THL on ladannut tai tulostanut ja jotka ovat THL:n tiedossa ja päätösten perustalla.

Jos saatavillanne ja käytössänne olevista lähteistä jokin vastaa yllä olevaa kuvausta ja on tällä hetkellä julkisesti saatavilla muualla, pyydän jokaisesta lähteestä riittävästi tietoa, jotta voin varmuudella löytää sen (esim. nimi, tekijä(t), päivämäärä, lehti, johon on julkinen pääsy). Pyydän myös URL-osoitteita mahdollisuuksien mukaan.
Pyydän dokumentteja pdf-muodossa sähköpostiin.

Yhteystiedot:

Sukunimi: Sipponen

Etunimi: Kaisa-Reetta

Osoite: Rillitie 4, 17200 Vääksy

Puhelinnumero: 044-2695999

Kiitos jo etukäteen!

感染研発第４８７号
令和３年７月２８日

行政文書不開示決定通知書

[redacted]

国立感染症研究所長　印

令和３年６月２７日付けの行政文書の開示請求（開第１８号）について、行政機関の保有する情報の公開に関する法律第９条第２項の規定に基づき、下記のとおり、開示しないことと決定しましたので通知します。

記

1　不開示決定した行政文書の名称
　1　新型コロナウイルスの存在を証明する科学的根拠、論文等
　2　PCR陽性判定の無症状者が、他者に新型コロナウイルスを感染させるという科学的根拠、論文等
　3　マスクの着用が新型コロナウイルスの感染防止に効果があるという科学的根拠、論文等
　4　新型コロナウイルスワクチンに効果があるという科学的根拠、論文等
　5　日本国は新型コロナウイルスワクチンが治験も終わってなく、安全、有効性も確立していない中、国民に接種させる科学的根拠、論文等

2　不開示とした理由
　開示請求に係る行政文書を保有していなかったため。

＊　この決定に不服がある場合は、行政不服審査法（平成26年法律第68号）の規定により、この決定があったことを知った日の翌日から起算して３か月以内に、厚生労働大臣に対して不服申立て（審査請求）をすることができます。（なお、決定があったことを知った日の翌日から起算して３か月以内であっても、決定の日の翌日から起算して１年を経過した場合には不服申立て（審査請求）をすることができなくなります。）

　また、この決定の取消しを求める訴訟を提起する場合は、行政事件訴訟法（昭和37年法律第139号）の規定により、この決定があったことを知った日から６か月以内に、国を被告として（訴訟において国を代表する者は法務大臣となります。）、東京地方裁判所又は特定管轄裁判所に処分の取消しの訴えを提起することができます。（なお、決定があったことを知った日から６か月以内であっても、決定の日から１年を経過した場合には処分の取消しの訴えを提起することができなくなります。）

令和３年１２月１４日

行政文書不開示決定通知書

[redacted]

国立感染症研究所長　印

令和３年１１月１５日付けの行政文書の開示請求（開第５０号）について、行政機関の保有する情報の公開に関する法律第９条第２項の規定に基づき、下記のとおり、開示しないことと決定しましたので通知します。

記

1　不開示決定した行政文書の名称

・日本国内に新型コロナウイルス(SARS-CoV2)が存在するのを立証する事ができる、ウイルスの標本、論文等のすべての開示

・新型コロナウイルス（SARS-CoV2）を分離した事を立証する事ができる論文等すべての開示

2　不開示とした理由

開示請求に係る行政文書を保有していなかったため。

＊　この決定に不服がある場合は、行政不服審査法(平成26年法律第68号)の規定により、この決定があったことを知った日の翌日から起算して３か月以内に、厚生労働大臣に対して不服申立て（審査請求）をすることができます。（なお、決定があったことを知った日の翌日から起算して３か月以内であっても、決定の日の翌日から起算して１年を経過した場合には不服申立て（審査請求）をすることができなくなります。）

また、この決定の取消しを求める訴訟を提起する場合は、行政事件訴訟法(昭和37年法律第139号)の規定により、この決定があったことを知った日から６か月以内に、国を被告として（訴訟において国を代表する者は法務大臣となります。）、東京地方裁判所又は特定管轄裁判所に処分の取消しの訴えを提起することができます。（なお、決定があったことを知った日から６か月以内であっても、決定の日から１年を経過した場合には処分の取消しの訴えを提起することができなくなります。）

＊　担当課等　総務部調整課　〒162-8640 新宿区戸山１－２３－１　TEL 03-5285-1111

行政文書不開示決定通知書

[redacted]

厚生労働大臣　　後藤　茂之

令和３年９月８日付け（同日受付）の行政文書の開示請求（開電第719号）について、行政機関の保有する情報の公開に関する法律（平成11年法律第42号。以下「法」という。）第９条第２項の規定に基づき、下記のとおり開示しないことと決定しましたので通知します。

記

1　不開示とした行政文書の名称

新型コロナウイルスワクチンに効果があるという科学的根拠、論文等。（製品に関する審査報告書は除く）

2　不開示とした理由

上記１の文書については、事務処理上作成又は取得した事実はなく、実際に保有していないため、不開示とした。

＊　この決定に不服がある場合は、行政不服審査法（平成26年法律第68号）の規定により、この決定があったことを知った日の翌日から起算して３月以内に、厚生労働大臣に対して審査請求をすることができます（決定があったことを知った日の翌日から起算して３月以内であっても、決定の日の翌日から起算して１年を経過した場合には審査請求をすることができなくなることに御注意ください。）。
　また、この決定の取消しを求める訴訟を提起する場合は、行政事件訴訟法（昭和37年法律第139号）の規定により、この決定があったことを知った日から６か月以内に、国を被告として（訴訟において国を代表する者は法務大臣となります。）、東京地方裁判所、処分庁管轄地方裁判所又は特定管轄裁判所に処分の取消しの訴えを提起することができます（決定があったことを知った日から６か月以内であっても、決定の日から１年を経過した場合には処分の取消しの訴えを提起することができなくなることに御注意ください。）。

3　担当課等

厚生労働省　健康局健康課予防接種室

TEL ： 03-5253-1111（内線　8165 ）

WOB-verzoek Netherlands

[redacted] Sat, Jan 22, 2022 at 7:42 AM

To: cmssyc@gmail.com

Dear Christine,

I wanted to inform you that I did a (short) WOB (FOI) request - by common mail, not e-mail - in the Netherlands after I had seen your interview with Tom Cowan on jan. 14.
I read that such a request was done already on a documentlist I found, but couldn't find any outcome.
So I did a try. Here is what it said. I translated it with Deepl into English.
It is said they should give me an answer within 4 weeks.
I will send the answer to you, if I get any.

"
Dear Minister Ernst Kuipers,

Could you please send me reliable evidence that SARS-CoV-2 (or any other so-called virus) has been isolated from an "unadulterated sample from a sick patient" (or a sick animal) and that that so-called virus can be transmitted to another person (or animal) and then cause the same so-called disease there?

I would like to receive from you the scientific evidence on which you base this and which unambiguously shows that the so-called virology is based on scientific facts.

I look forward to receiving your answer within the specified period.

Thank you in advance.
"

By the way, I did such a request earlier in my hometown to the mayor and to a health service (GGD) but did not call it a WOB request then. so I got evasive meaningless answers.
And I was aware of this scamdemic from day 1 and long before that.

Kind regards
[redacted]

WOB-verzoek Netherlands

[redacted] Thu, Mar 24, 2022 at 4:35 AM
To: Christine Massey <cmssyc@gmail.com>

Dear Christine,

My WOB-verzoek in the Netherlands has ended.

To summarize what happened:

On januari 14 I send the request. (WOB verzoek virologie)
On januari 31 they send me the answer in which they stated that I did (only) a covid-19 request, which I did not. And they took 4 weeks more to answer. (WOB VWS 01)

I tried to send them an e-mail with the e-mailaddress in the letter. I tried several versions because the emailaddress was not clear, but they all came back.
So I searched on the internet to contact them by mail, I found a contact form and asked in it for the right e-mailaddress of VWS. What came back was: We send your question to VWS. But I didn't hear anything back. A good week later I tried again and again the same thing. So I let it and waited.

On march 15 they send me their answer in which they gave me 2 url's, I briefly read them and saw they did not apply to my request. (WOB VWS 02)
On march 22 I send them my answer in which I told them they failed to give me any scientific proof. (WOB verzoek virologie 2)

I guess this has ended now, but if I get any respons again I will send it to you.

Kind regards,
[redacted]

By the way, when I heard your surname, I immediately thought of Gerald Massey who wrote about ancient Egypt. The Light of the World and other works. (I just know his work very briefly, mainly by the work of Alvin Boyd Kuhn)

I guess you should be related to him... ;)

Truth is all-potent with its silent power
If only whispered, never heard aloud,
But working secretly, almost unseen,
Save in some excommunicated Book;
'Tis as the lightning with its errand done
Before you hear the thunder.

(from the prefatory of the Light of the World)

4 attachments

WOB-VWS_01.pdf
1234K

WOB-VWS_02.pdf
768K

WOB-verzoek-virologie.pdf
18K

WOB-verzoek-virologie-2.pdf
28K

Ministerie van Volksgezondheid, Welzijn en Sport
t.a.v. minister Ernst Kuipers
Parnassusplein 5
2511 VX Den Haag

14 januari 2022

WOB-verzoek

Beste minister Ernst Kuipers,

Kunt u mij betrouwbaar bewijs toesturen dat SARS-CoV-2 (of enig ander zogenaamd virus) geïsoleerd is uit een "onvervalst monster van een zieke patiënt" (of een ziek dier) en dat dat zogenaamde virus kan worden overgedragen op een ander persoon (of dier) en er vervolgens een zelfde zogenaamde ziekte veroorzaakt?

Graag ontvang ik van u het wetenschappelijke bewijs waarop u dit baseert en waaruit eenduidig blijkt dat de zogenaamde virologie op wetenschappelijke feiten berust.

Ik zie uw antwoord graag binnen de gestelde termijn tegemoet.

Bijvoorbaat dank.

Vriendelijke groet,
[redacted]

verblijf:
[redacted]

Ministerie van Volksgezondheid,
Welzijn en Sport

> Retouradres Postbus 20350 2500 EJ Den Haag

Programmadirectie Openbaarheid
Bezoekadres:
Parnassusplein 5
2511 VX Den Haag
T 070 340 79 11
F 070 340 59 84
www.rijksoverheid.nl

Inlichtingen bij
Balie PDO
E _dienstpostbusWob-corona-ondersteuning@minvws.nl
T 070 340 60 80

Ons kenmerk
2022.023/001

Bijlage(n)
-

Uw brief
14 januari 2022

Correspondentie uitsluitend richten aan het retouradres met vermelding van de datum en het kenmerk van deze brief.

Datum: 31 januari 2022
Betreft: Ontvangstbevestiging van uw verzoek om informatie

Geachte heer/mevrouw Kabboord,

Op 18 januari 2022 ontving ik uw brief van 14 januari 2022 waarin u met een beroep op de Wet openbaarheid van bestuur (hierna: de Wob) vraagt om openbaarmaking van documenten over het coronavirus. Uw verzoek heb ik geregistreerd onder nummer 2022.023. De behandeling van uw Wob-verzoek gaat via een afwijkende werkwijze. Ik wil u daar door middel van deze brief over informeren.

De inzet van mijn medewerkers, van de medewerkers van het Rijksinstituut Volksgezondheid en Milieu (RIVM) en van de medewerkers van de Inspectie Gezondheidszorg en Jeugd (IGJ), heeft ertoe geleid dat zich onder mijn ministerie uitzonderlijk veel informatie bevindt over het coronavirus en over de bestrijding ervan. Net als u, zijn er veel mensen die om de openbaarmaking van deze informatie verzoeken. Om in deze grote informatiebehoefte te kunnen voorzien, ben ik genoodzaakt een afwijkend werkproces aan te houden. In plaats van ieder Wob-verzoek apart in behandeling te nemen, is ervoor gekozen zo veel mogelijk informatie per deelonderwerp gefaseerd voor iedereen openbaar te maken. Door het instellen van deze afwijkende werkwijze kan toch worden voldaan aan het met de Wob gediende zwaarwegende belang van openbaarheid.

De openbaarmaking van de door u gevraagde informatie over 'bewijs SARS-Covid-19' is in het afwijkende werkproces opgenomen. Deze informatie wordt gefaseerd openbaargemaakt met de openbaarmaking van informatie inzake het deelonderwerp RIVM. Zodra de informatie is gepubliceerd zult u geïnformeerd worden over de vindplaats. Ik hoop dat ik kan rekenen op uw begrip voor deze afwijkende werkwijze.

In verband met de grote hoeveelheid openbaar te maken documenten lukt het mij niet om binnen vier weken een besluit te nemen op uw verzoek. Ik verleng daarom de beslistermijn voor uw verzoek op grond van artikel 6, tweede lid, van de Wob, met vier weken.

Mocht u naar aanleiding van deze brief nog vragen hebben over de behandeling van uw Wob-verzoek, neemt u dan gerust contact op met het Wob-cluster. U kunt daarvoor gebruik maken van het hiervoor vermelde e-mailadres.

Programmadirectie Openbaarheid

Ons kenmerk
2022.023/001

Hoogachtend,

de Minister van Volksgezondheid,
Welzijn en Sport,
namens deze,
clusterhoofd directie Wetgeving en Juridische Zaken,

mr. C. Visser

> Retouradres Postbus 20350 2500 EJ Den Haag

[redacted]

Programmadirectie Openbaarheid
Bezoekadres:
Parnassusplein 5
2511 VX Den Haag
T 070 340 79 11
F 070 340 59 84
www.rijksoverheid.nl

Inlichtingen bij
mr. F. el Benaissati

Cluster Wob
E _dienstpostbusWob-corona-ondersteunino@minvws.nl
T 070 340 60 80

Ons kenmerk
2022.023
3334863-1026144-WJZ

Uw brief
14 januari 2022

Correspondentie uitsluitend richten aan het retouradres met vermelding van de datum en het kenmerk van deze brief.

Datum: 15 MRT 2022
Betreft: Verzoek om informatie inzake het bestaan van Sars-Cov2

Geachte heer [redacted]

Op 18 januari 2022 ontving ik uw brief van 14 januari 2022. De ontvangst hiervan heb ik bevestigd bij brief van 31 januari 2022 met kenmerk 2022.023/001.

Met een beroep op de Wet openbaarheid van bestuur (hierna: Wob) verzoekt u om wetenschappelijk bewijs van het bestaan van het Sars-Cov2-virus.

De plicht tot openbaarmaking op grond van de Wob heeft geen betrekking op informatie die reeds openbaar is. De door u gevraagde informatie is al openbaar en ik zal hieronder aangeven waar u dit kunt vinden.

Het coronavirus Sars-Cov-2 is het virus dat de ziekte COVID-19 kan veroorzaken. Meer informatie hierover kunt u vinden op de site van het RIVM onder het kopje 'virus' (www.rivm.nl/coronavirus-covid-19/virus). Voor de wetenschappelijke rapporten over het bestaan van Sars-Cov2 verwijs ik u naar de volgende sites: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7159086/ en https://pubmed.ncbi.nlm.nih.gov/31978945/.

Mocht u nog vragen hebben over deze brief, neemt u dan gerust contact op met de Programmadirectie Openbaarheid. De contactgegevens staan bovenaan deze brief.

Hoogachtend,

de Minister van Volksgezondheid,
Welzijn en Sport,
namens deze,
de directeur Programmadirectie Openbaarheid,

mr. C.A. Grezel

Ministerie van Volksgezondheid, Welzijn en Sport
t.a.v. mr. C.A. Grezel
Postbus 20350
2500 EJ Den Haag

22 maart 2022

WOB-verzoek

Beste mr. C.A. Grezel,

Dank voor uw brief van 15 maart 2022.

Ik vroeg u in mijn verzoek van 14 januari 2022 mij betrouwbaar bewijs toe te sturen dat SARS-CoV-2 (of enig ander zogenaamd virus) geïsoleerd is uit een "onvervalst monster van een zieke patiënt" (of een ziek dier) en dat dat zogenaamde virus kan worden overgedragen op een ander persoon (of dier) en er vervolgens een zelfde zogenaamde ziekte veroorzaakt.

Dit blijkt niet uit hetgeen u mij heeft aangereikt. Te weten:
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7159086/ en
https://pubmed.ncbi.nlm.nih.gov/31978945/

Het via **onzuivere** kweeks gedeeltelijke **in silico** sequenties verkrijgen en daarmee vervolgens een zogenaamd bestaan van een (al of niet nieuw) virus aantonen *staat niet gelijk aan* het op zuivere wijze wetenschappelijk isoleren uit een onvervalst monster van een zieke patiënt en het bestaan van een besmettelijk virus aantonen.

Ik moet dus – voor u helaas – concluderen dat u in gebreke bent gebleven en geen wetenschappelijk bewijs heeft voor het bestaan van enig virus. Waarvan akte.

Vriendelijke groet,
[redacted]

verblijf:
[redacted]