[redacted]

To: FOIA Requests (CDC)
Subject: FOIA: Control Group Information requested for Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States
Date: Sunday, August 1, 2021 7:44:01 PM

Dear Freedom of Information Officer,

This is a formal request for access to general records, made under the Freedom of Information Act.

Description of Requested Records

I am requesting control group information about the publication listed on the CDC's web site named, "Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States" with a url of https://wwwnc.cdc.gov/eid/article/26/6/20-0516_article.

I understand that several of the authors listed on this paper are employed by the CDC and so the CDC should have access to this additional information.

Control Group Information:

- Did the scientist for this paper use control groups?
- If so, did the control groups use the same formulations of cell culture mixtures as the experimental groups sans the sample containing the alleged viruses?
- For instance, the experimental groups contained the following contents at the specified volumes:
    - viral transport medium
    - 2× penicillin/streptomycin
    - 2× antibiotics/antimycotics
    - 2× amphotericin B
    - 10% fetal bovine serum
- Did the control groups use the same volume and type of nutrient solution?
    - For instance, Dulbecco minimal essential medium (DMEM)

In summary, if control groups were used, please list details of the control groups.

I don't think this is a complex request and should be readily available at the CDC.

Thank you
--

[redacted]

To: Thomas, Paula (CDC/OCOO/OD)
Subject: Re: Your CDC FOIA Request #21-01704-FOIA
Date: Tuesday, August 3, 2021 4:18:59 PM

Greetings,

Here is my modified request:

This is a request for all supplemental records, laboratory notes, and control group details for the following paper: "Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States" with a url of https://wwwnc.cdc.gov/eid/article/26/6/20-0516_article.

Regards

On Tue, Aug 3, 2021 at 11:32 PM <PThomas3@cdc.gov> wrote:

> August 3, 2021
>
> Request Number: 21-01704-FOIA
>
> [redacted]
>
> This is regarding your Freedom of Information Act (FOIA) request of August 2, 2021, for request for control group information about the publication listed on the CDC's web site named, "Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States" with a url of https://wwwnc.cdc.gov/eid/article/26/6/20-0516_article. Control Group Information: • Did the scientist for this paper use control groups? • If so, did the control groups use the same formulations of cell culture mixtures as the experimental groups sans the sample containing the alleged viruses? • For instance, the experimental groups contained the following contents at the specified volumes: o viral transport medium o 2× penicillin/streptomycin o 2× antibiotics/antimycotics o 2× amphotericin B o 10% fetal bovine serum • Did the control groups use the same volume and type of nutrient solution? o For instance, Dulbecco minimal essential medium (DMEM) In summary, if control groups were used, please list details of the control groups..
>
> Please see the attached letter.
>
> Sincerely,
>
> CDC/ATSDR FOIA Office
> 770-488-6399

NATIONAL ARCHIVES and RECORDS ADMINISTRATION
8601 ADELPHI ROAD - OGIS | COLLEGE PARK, MD 20740-6001
www.archives.gov/ogis | ogis@nara.gov | o: 202.741.5770 | f: 202.741.5769 | t: 877.684.6448

March 8, 2022—Sent via email

[redacted]

[redacted]

Thank you for contacting the Office of Government Information Services (OGIS). As you are aware, Congress created OGIS to serve as the federal Freedom of Information Act (FOIA) Ombudsman. We assist the public and federal agencies by helping them resolve their FOIA disputes, and by addressing their questions and concerns about the FOIA process.

We understand that you seek assistance obtaining the status of a FOIA request you submitted to the Centers for Disease Control and Prevention (CDC). We contacted the agency on your behalf regarding this matter. CDC FOIA officials informed us that they anticipate responding to **21-01704-FOIA** in April 2022. Please note, due to the COVID-19 outbreak many agencies have moved to telework. While this allows agencies to continue to process some FOIA requests, agencies may experience unanticipated delays in responding to requests because of unforeseen complications related to the pandemic.

We hope you find this information useful. At this time, we will take no further action. If you have questions or concerns that we have not addressed, please contact us again.

Best regards,
The OGIS Staff

DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service

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

March 29, 2022

SENT VIA EMAIL

[redacted]

[redacted]

This letter is regarding your Centers for Disease Control and Prevention and Agency for Toxic Substances and Disease Registry (CDC/ATSDR) Freedom of Information Act (FOIA) request of August 2, 2021, assigned #21-01704-FOIA.

We located 37 pages of responsive records and one excel spreadsheet (37 pages released in full or part; no pages withheld in full). After a careful review of these pages, some information was withheld from release pursuant to 5 U.S.C. §552 Exemption 6.

Exemption 6 protects information in personnel and medical files and similar files when disclosure would constitute a clearly unwarranted invasion of personal privacy. The information that has been withheld under Exemption 6 consists of personal information, such as cell phone numbers and scope picture identification numbers. We have determined that the individuals to whom this information pertains has a substantial privacy interest in withholding it.

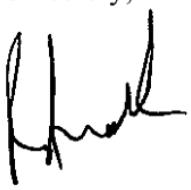
In accordance with the Department's implementing regulations, 45 CFR Part 5, no FOIA processing fees are due for your request.

You may contact our FOIA Public Liaison at 770-488-6246 for any further assistance and to discuss any aspect of your request. Additionally, you may contact the Office of Government Information Services (OGIS) at the National Archives and Records Administration to inquire about the FOIA mediation services they offer. The contact information for OGIS is as follows: Office of Government Information Services, National Archives and Records Administration, 8601 Adelphi Road-OGIS, College Park, Maryland 20740-6001, e-mail at ogis@nara.gov; telephone at 202-741-5770; toll free at 1-877-684-6448; or facsimile at 202-741-5769.

[redacted]

If you are not satisfied with the response to this request, you may administratively appeal to the Deputy Agency Chief FOIA Officer, Office of the Assistant Secretary for Public Affairs, U.S. Department of Health and Human Services, via the online portal at https://requests.publiclink.hhs.gov/App/Index.aspx. Your appeal must be electronically transmitted by Monday, June 27, 2022.

Sincerely,



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

2 Enclosures:

1. CDC Responsive Records
2. Original FOIA Request

#21-01704-FOIA

From: Queen, Krista (CDC/DDID/NCIRD/DVD)
Sent: Wed, 5 Feb 2020 13:06:54 +0000
To: Thornburg, Natalie (CDC/DDID/NCIRD/DVD); Harcourt, Jennifer (CDC/DDID/NCIRD/DVD); Tamin, Azaibi (CDC/DDID/NCIRD/DVD)
Subject: FW: GenBank MT020880-MT020881

Good morning,
Here are the GenBank accession numbers for the two isolates. Thanks!

Krista
-----Original Message-----
From: gb-admin@ncbi.nlm.nih.gov <gb-admin@ncbi.nlm.nih.gov>
Sent: Wednesday, February 5, 2020 8:04 AM
To: Tong, Suxiang (Sue) (CDC/DDID/NCIRD/DVD) <sot1@cdc.gov>; Queen, Krista (CDC/DDID/NCIRD/DVD) <wyz0@cdc.gov>
Subject: GenBank MT020880-MT020881

Dear GenBank Submitter:

Thank you for your direct submission of sequence data to GenBank. We have provided GenBank accession numbers for your nucleotide sequences:

BankIt2310056 2019-nCoV/USA-WA1-A12/2020 MT020880
BankIt2310056 2019-nCoV/USA-WA1-F6/2020 MT020881

The GenBank accession numbers should appear in any publication that reports or discusses these data, as it gives the community a unique label with which they may retrieve your data from our on-line servers. You may prepare and submit your manuscript before your accessions are released in GenBank.

Submissions are not automatically deposited into GenBank after being accessioned. Each sequence record is individually examined and processed by the GenBank annotation staff to ensure that it is free of errors or problems.

You have not requested a specific release date for your sequence data.
Therefore, your record(s) will be released to the public database once they are processed. If this is not what you intended, please contact us as soon as possible with the correct release date.

Since the flatfile record is a display format only and is not an editable format of the data, do not make changes directly to a flatfile. For complete information about different methods to update a sequence record, see: https://www.ncbi.nlm.nih.gov/Genbank/update.html

Any inquiries about your submission should be sent to gb-admin@ncbi.nlm.nih.gov

For more information about the submission process or the available submission tools, please contact GenBank User Support at info@ncbi.nlm.nih.gov.

Please reply using the current Subject line.

Sincerely,

Linda Yankie, PhD

The GenBank Direct Submission Staff
Bethesda, Maryland USA
****
gb-admin@ncbi.nlm.nih.gov (for updates/replies to GenBank entries)
info@ncbi.nlm.nih.gov (for general questions regarding GenBank)
****

From: Tamin, Azaibi (CDC/DDID/NCIRD/DVD)
Sent: Mon, 27 Jan 2020 18:53:13 +0000
To: Goldsmith, Cynthia (CDC/DDID/NCEZID/DHCPP)
Cc: Thornburg, Natalie (CDC/DDID/NCIRD/DVD); Zaki, Sherif (CDC/DDID/NCEZID/DHCPP)
Subject: FW: RT-PCR confirmation for 2 potential isolates of 2019 N CoV
Attachments: 2019 NCoV_2 potential isolates from the 1st US case.pdf

Hi Cynthia,
We have potential isolates of 2019 N-CoV from the first US case. While we are waiting for molecular confirmation, and in the meantime I'm scaling it up for higher volume/titer. What's your availability for EM study? Would T-25 or T-75 flask culture would be sufficient?

Thank you,
AT

Azaibi Tamin, Ph.D.
Research Microbiologist,
Respiratory Viruses Imunology Team, Respiratory Viruses Branch,
Division of Viral Diseases, National Center for Immunization and Respiratory Disease,
Centers for Disease Control and Prevention, Atlanta GA 30333, USA
Email: atamin@cdc.gov Tel: (404) 639 1302 Cell: (b)(6)

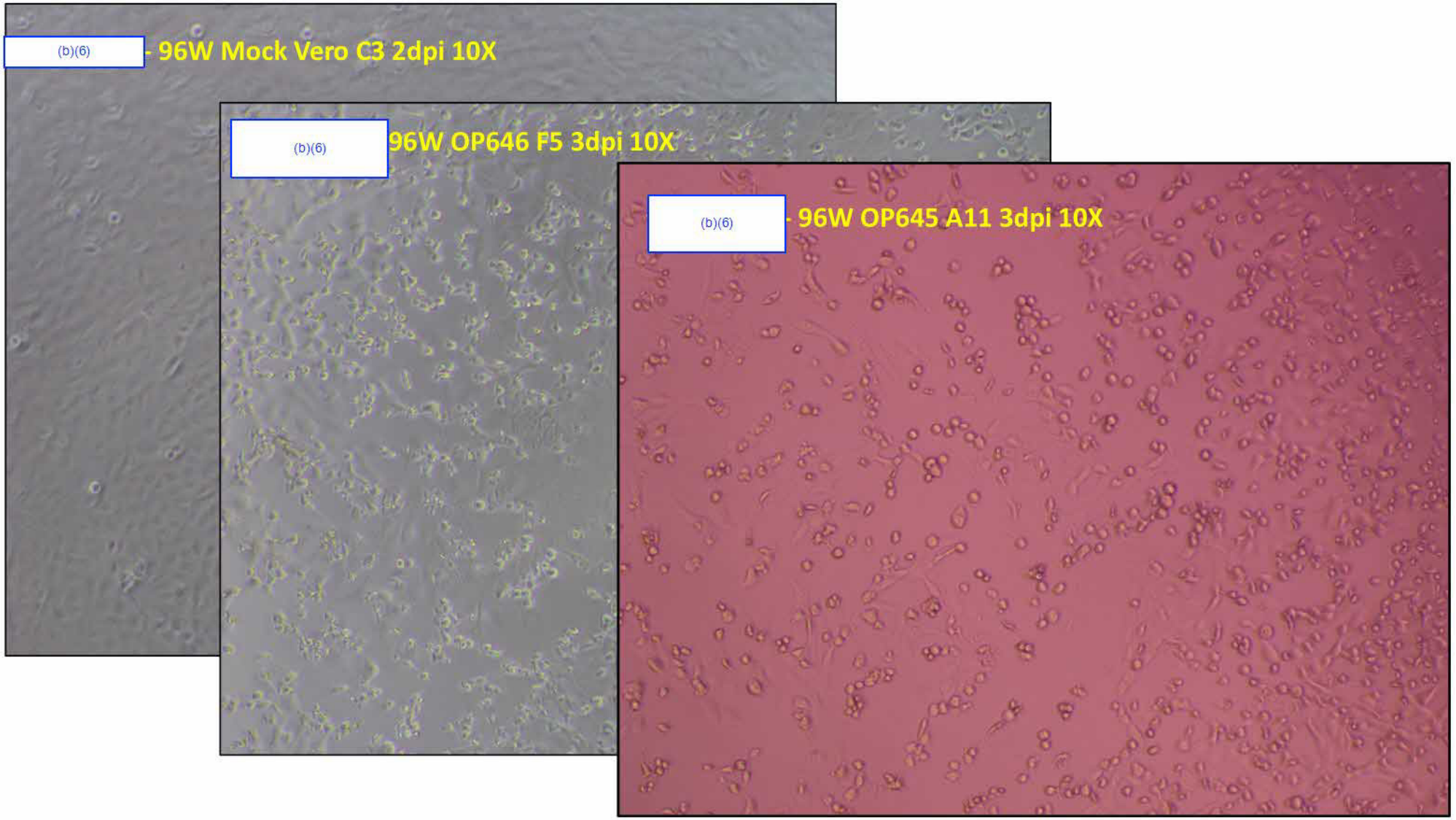
From: Tamin, Azaibi (CDC/DDID/NCIRD/DVD)
Sent: Monday, January 27, 2020 1:43 PM
To: Tong, Suxiang (Sue) (CDC/DDID/NCIRD/DVD) <sot1@cdc.gov>; Queen, Krista (CDC/DDID/NCIRD/DVD) <wyz0@cdc.gov>
Cc: Thornburg, Natalie (CDC/DDID/NCIRD/DVD) <nax3@cdc.gov>; Harcourt, Jennifer (CDC/DDID/NCIRD/DVD) <zaq6@cdc.gov>
Subject: RT-PCR confirmation for 2 potential isolates of 2019 N CoV

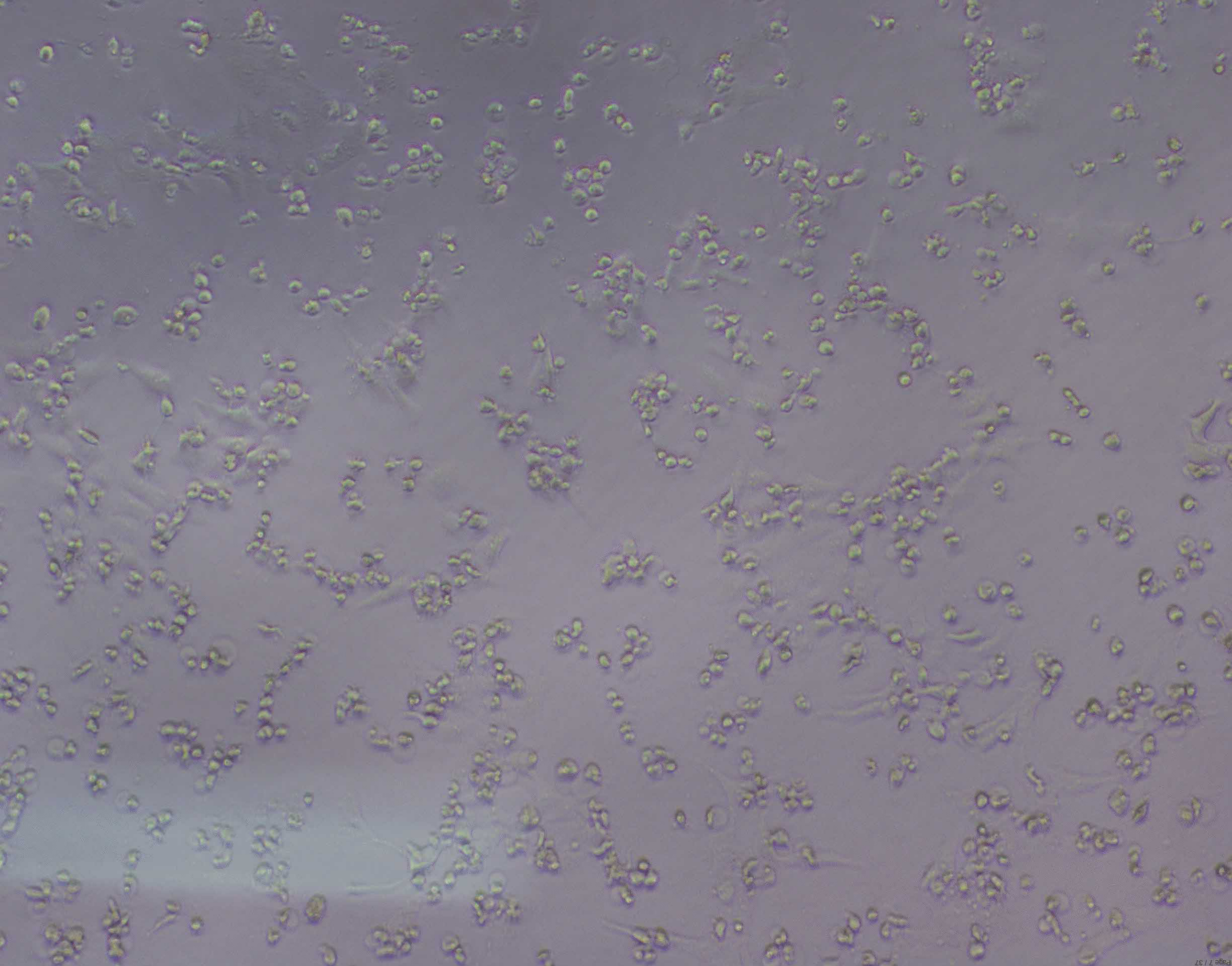
Hi Sue and Krista,
Jennifer Harcourt have passed to you 2 lysates (passage 1) from the case #1 US case, in AVL lysis buffer (350 uls AVL + 50 uls virus lysates) for molecular confirmation. Attached are their scope pics at 3 days post infection. Thank you for your time and help.

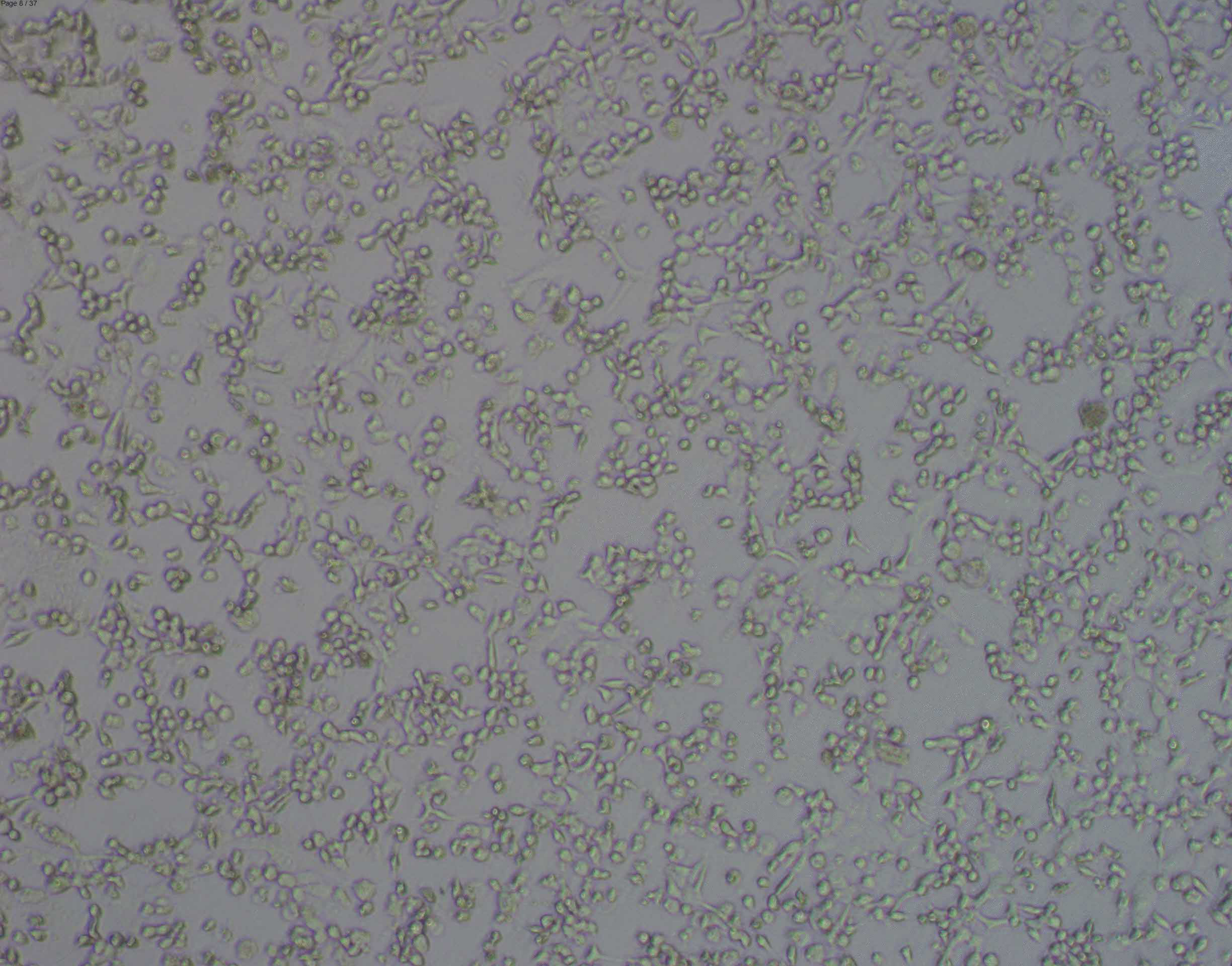
Cheers,
AT

Azaibi Tamin, Ph.D.

Research Microbiologist,
Respiratory Viruses Imunology Team, Respiratory Viruses Branch,
Division of Viral Diseases, National Center for Immunization and Respiratory Disease,
Centers for Disease Control and Prevention, Atlanta GA 30333, USA
Email: atamin@cdc.gov Tel: (404) 639 1302 Cell: (b)(6)


(b)(6)
96W Mock Vero C3 2dpi 10X
(b)(6)
96W OP646 F5 3dpi 10X
(b)(6)
96W OP645 A11 3dpi 10X





"For administrative convenience and to fully respond to your request, program staff have provided the following information below with corresponding web links.
The responses below address the isolation of the virus from the diseased host, which requires growth in cell culture. Viruses do not replicate outside of a host or in a pure culture (devoid of other cells). Koch's postulates were formed prior to the identification of viruses as the causative agents of some diseases and also pre-date modern microbiological techniques, including the ability to isolate viruses from hosts. As such, Koch's postulates have limitations when evaluating viruses and do not adequately account for the way viruses are isolated and propagated given that viruses are obligate intracellular parasites.

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.

Read more about CDC's work to culture the SARS-CoV-2 virus: 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 1 million sequences."

From: Tamin, Azaibi (CDC/DDID/NCIRD/DVD)
Sent: Tue, 4 Feb 2020 14:11:28 +0000
To: Thornburg, Natalie (CDC/DDID/NCIRD/DVD)
Subject: RE: cpe pictures
Attachments: (b)(6) - 96W Mock Vero C2 2dpi 10X.TIF, (b)(6) - 96W OP645 A12 3dpi 10X.TIF (b)(6) - 24W (b)(6) P2 2dpi 10X.TIF

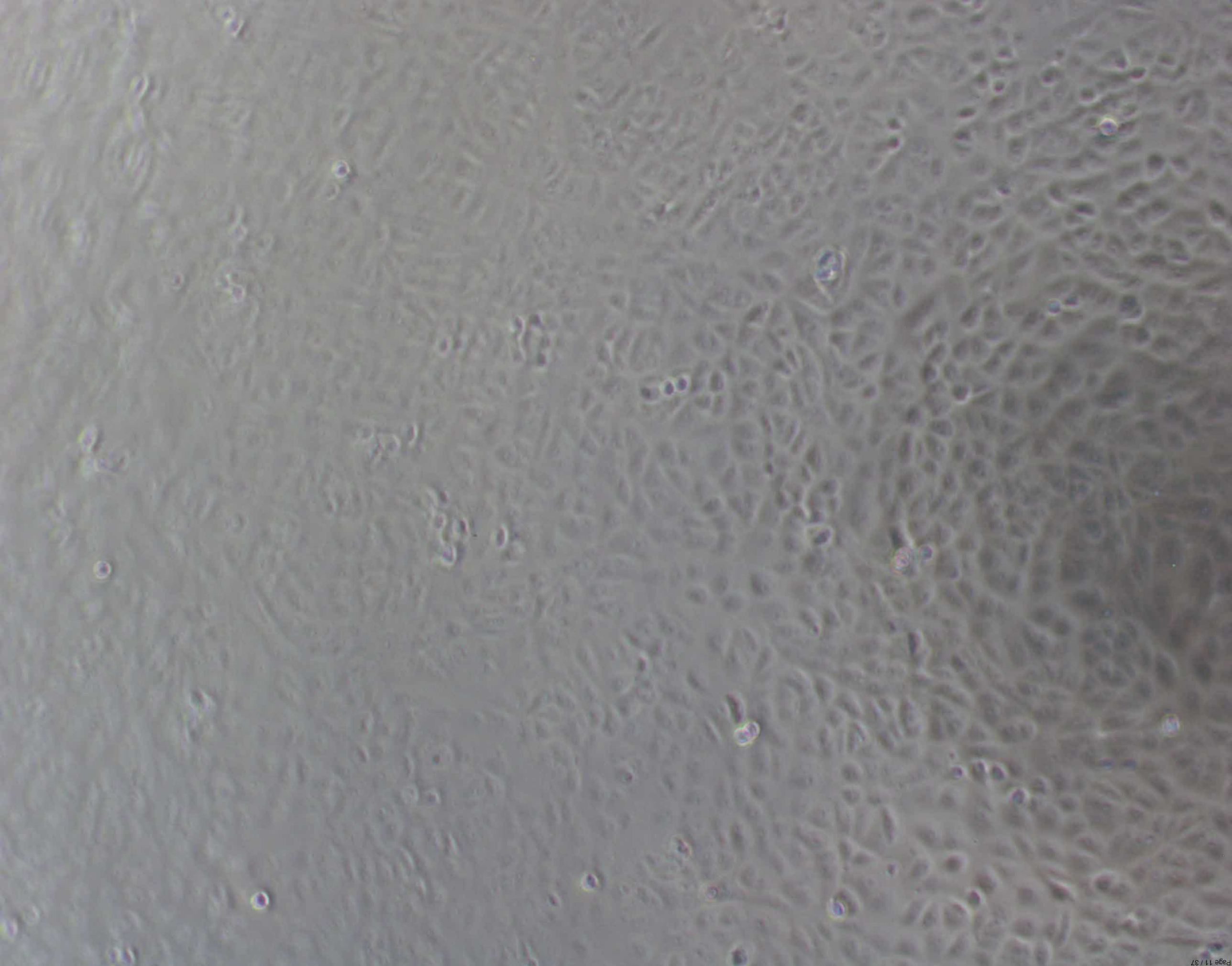
These are CPE scope pics for Passage 1 (96W plate), and passage 2 (24W plate) of the (b)(6) solate that was tested for confirmation and exclusive testing. Will shoot the P3 in T75 later.

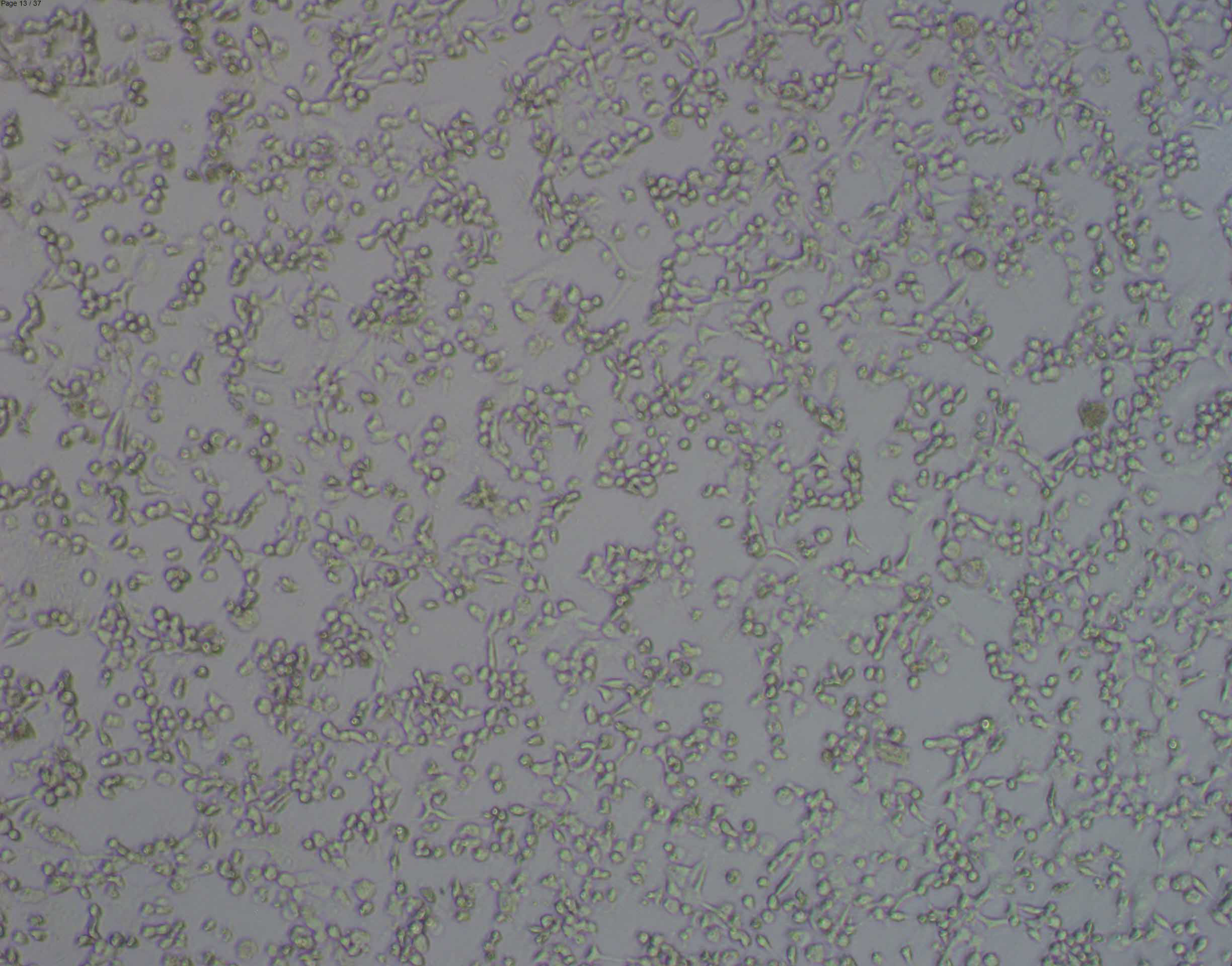
From: Thornburg, Natalie (CDC/DDID/NCIRD/DVD) <nax3@cdc.gov>
Sent: Monday, February 3, 2020 2:02 PM
To: Tamin, Azaibi (CDC/DDID/NCIRD/DVD) <axt4@cdc.gov>
Subject: cpe pictures

Could you send the original JPEG or TIFF files for your CPE images? I want to start working on a publication quality figure.

Natalie J. Thornburg, Ph.D.
Respiratory virus immunology team lead
Division of Viral Diseases
National Center for Immunization and Respiratory Diseases

Centers for Disease Control and Prevention (CDC)
1600 Clifton Road, NE, Mailstop G-18, Atlanta, GA 30329
404.639.3797 Office | (b)(6) Work Cell | nax3@cdc.gov





From: Gautam, Rashi (CDC/DDID/NCIRD/DVD)
Sent: Fri, 31 Jan 2020 19:57:42 +0000
To: Thornburg, Natalie (CDC/DDID/NCIRD/DVD); Murray, Janna' R. (CDC/DDID/NCIRD/DVD) (CTR)
Cc: Queen, Krista (CDC/DDID/NCIRD/DVD); Tong, Suxiang (Sue) (CDC/DDID/NCIRD/DVD); Harcourt, Jennifer (CDC/DDID/NCIRD/DVD)
Subject: RE: Real-time PCR results for virus samples submitted

Great, please let me know if you want me to test any more extracts by FTD kit.

From: Thornburg, Natalie (CDC/DDID/NCIRD/DVD) <nax3@cdc.gov>
Sent: Friday, January 31, 2020 2:56 PM
To: Gautam, Rashi (CDC/DDID/NCIRD/DVD) <ijs0@cdc.gov>; Murray, Janna' R. (CDC/DDID/NCIRD/DVD) (CTR) <hko3@cdc.gov>
Cc: Queen, Krista (CDC/DDID/NCIRD/DVD) <wyz0@cdc.gov>; Tong, Suxiang (Sue) (CDC/DDID/NCIRD/DVD) <sot1@cdc.gov>; Harcourt, Jennifer (CDC/DDID/NCIRD/DVD) <zaq6@cdc.gov>
Subject: RE: Real-time PCR results for virus samples submitted

Hooray, thank you so much! We have our ducks in a row to start shipping some virus out next week.

From: Gautam, Rashi (CDC/DDID/NCIRD/DVD) <ijs0@cdc.gov>
Sent: Friday, January 31, 2020 2:55 PM
To: Thornburg, Natalie (CDC/DDID/NCIRD/DVD) <nax3@cdc.gov>; Murray, Janna' R. (CDC/DDID/NCIRD/DVD) (CTR) <hko3@cdc.gov>
Cc: Queen, Krista (CDC/DDID/NCIRD/DVD) <wyz0@cdc.gov>; Tong, Suxiang (Sue) (CDC/DDID/NCIRD/DVD) <sot1@cdc.gov>; Harcourt, Jennifer (CDC/DDID/NCIRD/DVD) <zaq6@cdc.gov>
Subject: RE: Real-time PCR results for virus samples submitted

Hi Natalie,
I had tested 4 extracts (A12, B10, E4 and F6) using FTD 33 kit, attached is the labelled run file and an excel table with Ct values. All the positive controls showed positive results except nFTD IC(EAV) as no internal control was used in this experiment. All the 4 extracts have tested negative with all 8 mastermixes.
Please let me know if you have any questions.
Thanks
Rashi

From: Thornburg, Natalie (CDC/DDID/NCIRD/DVD) <nax3@cdc.gov>
Sent: Thursday, January 30, 2020 8:47 AM
To: Murray, Janna' R. (CDC/DDID/NCIRD/DVD) (CTR) <hko3@cdc.gov>
Cc: Queen, Krista (CDC/DDID/NCIRD/DVD) <wyz0@cdc.gov>; Tong, Suxiang (Sue) (CDC/DDID/NCIRD/DVD) <sot1@cdc.gov>; Harcourt, Jennifer (CDC/DDID/NCIRD/DVD) <zaq6@cdc.gov>; Gautam, Rashi (CDC/DDID/NCIRD/DVD) <ijs0@cdc.gov>
Subject: RE: Real-time PCR results for virus samples submitted

Rashi,
Janna on Steve's team did RNA extractions yesterday, and she has leftovers for fast track assay. You can get them from her anytime now.

Thank you again,

Natalie

From: Murray, Janna' R. (CDC/DDID/NCIRD/DVD) (CTR) <hko3@cdc.gov>
Sent: Wednesday, January 29, 2020 4:49 PM
To: Tamin, Azaibi (CDC/DDID/NCIRD/DVD) <axt4@cdc.gov>; Thornburg, Natalie (CDC/DDID/NCIRD/DVD) <nax3@cdc.gov>
Cc: Lindstrom, Stephen (CDC/DDID/NCIRD/DVD) <sql5@cdc.gov>
Subject: Real-time PCR results for virus samples submitted

Hi Azaibi and Natalie,

Attached are the Real-time PCR results for the 4 viruses you submitted for testing. Each sample was tested in triplicate so there are 3 Ct values for each. The samples are named according to what was written on the tube. Let me know if you have any questions.

Regards,
Janná

Janná Murray, MPH
Microbiologist (Eagle Contractor)
Respiratory Viruses Diagnostic Laboratory
Respiratory Viruses Branch
Division of Viral Diseases, NCIRD
Centers for Disease Control and Prevention, CDC
1600 Clifton Road NE, MS-G04 Atlanta, GA 30329-4027
Office: 404-639-0134
jrmurray@cdc.gov

From: Goldsmith, Cynthia (CDC/DDID/NCEZID/DHCPP)
Sent: Mon, 27 Jan 2020 19:09:29 +0000
To: Tamin, Azaibi (CDC/DDID/NCIRD/DVD)
Cc: Thornburg, Natalie (CDC/DDID/NCIRD/DVD); Zaki, Sherif (CDC/DDID/NCEZID/DHCPP); Martines, Roosecelis (Rose) (CDC/DDID/NCEZID/DHCPP); Bullock, Hannah (CDC/DDID/NCEZID/DHCPP) (CTR)
Subject: RE: RT-PCR confirmation for 2 potential isolates of 2019 N CoV
Attachments: Zhu 2020 NEJM NCoV-2019 Wuhan.pdf

Azaibi,

That is really great that you have an isolate from the first US case! I believe that a T-25 flask (or perhaps a pooling of two T-25 flasks) would be good for thin section EM, and we would also like some fixed supernatant for a negative stain sample.. We are available to work on this whenever you have it ready, just let me bring you some fresh fixatives.

I am looping in Roose (our team lead) and Hannah (electron microscopist).

You may already be aware of the NEJM article (attached) which has 2 very nice EM images in figure 3, one from "human airway epithelial".

Thank you,
Cynthia

From: Tamin, Azaibi (CDC/DDID/NCIRD/DVD) <axt4@cdc.gov>
Sent: Monday, January 27, 2020 1:53 PM
To: Goldsmith, Cynthia (CDC/DDID/NCEZID/DHCPP) <csg1@cdc.gov>
Cc: Thornburg, Natalie (CDC/DDID/NCIRD/DVD) <nax3@cdc.gov>; Zaki, Sherif (CDC/DDID/NCEZID/DHCPP) <sxz1@cdc.gov>
Subject: FW: RT-PCR confirmation for 2 potential isolates of 2019 N CoV

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Thank you,
AT

Azaibi Tamin, Ph.D.
Research Microbiologist,
Respiratory Viruses Imunology Team, Respiratory Viruses Branch,
Division of Viral Diseases, National Center for Immunization and Respiratory Disease,

Centers for Disease Control and Prevention, Atlanta GA 30333, USA
Email: atamin@cdc.gov Tel: (404) 639 1302 Cell: (b)(6)

From: Tamin, Azaibi (CDC/DDID/NCIRD/DVD)
Sent: Monday, January 27, 2020 1:43 PM
To: Tong, Suxiang (Sue) (CDC/DDID/NCIRD/DVD) <sot1@cdc.gov>; Queen, Krista (CDC/DDID/NCIRD/DVD) <wyz0@cdc.gov>
Cc: Thornburg, Natalie (CDC/DDID/NCIRD/DVD) <nax3@cdc.gov>; Harcourt, Jennifer (CDC/DDID/NCIRD/DVD) <zaq6@cdc.gov>
Subject: RT-PCR confirmation for 2 potential isolates of 2019 N CoV

Hi Sue and Krista,
Jennifer Harcourt have passed to you 2 lysates (passage 1) from the case #1 US case, in AVL lysis buffer (350 uls AVL + 50 uls virus lysates) for molecular confirmation. Attached are their scope pics at 3 days post infection. Thank you for your time and help.

Cheers,
AT

Azaibi Tamin, Ph.D.
Research Microbiologist,
Respiratory Viruses Imunology Team, Respiratory Viruses Branch,
Division of Viral Diseases, National Center for Immunization and Respiratory Disease,
Centers for Disease Control and Prevention, Atlanta GA 30333, USA
Email: atamin@cdc.gov Tel: (404) 639 1302 Cell: (b)(6)

The NEW ENGLAND JOURNAL of MEDICINE

BRIEF REPORT

A Novel Coronavirus from Patients with Pneumonia in China, 2019

Na Zhu, Ph.D., Dingyu Zhang, M.D., Wenling Wang, Ph.D., Xinwang Li, M.D., Bo Yang, M.S., Jingdong Song, Ph.D., Xiang Zhao, Ph.D., Baoying Huang, Ph.D., Weifeng Shi, Ph.D., Roujian Lu, M.D., Peihua Niu, Ph.D., Faxian Zhan, Ph.D., Xuejun Ma, Ph.D., Dayan Wang, Ph.D., Wenbo Xu, M.D., Guizhen Wu, M.D., George F. Gao, D.Phil., and Wenjie Tan, M.D., Ph.D., for the China Novel Coronavirus Investigating and Research Team

SUMMARY

In December 2019, a cluster of patients with pneumonia of unknown cause was linked to a seafood wholesale market in Wuhan, China. A previously unknown betacoronavirus was discovered through the use of unbiased sequencing in samples from patients with pneumonia. Human airway epithelial cells were used to isolate a novel coronavirus, named 2019-nCoV, which formed another clade within the subgenus sarbecovirus, Orthocoronavirinae subfamily. Different from both MERS-CoV and SARS-CoV, 2019-nCoV is the seventh member of the family of coronaviruses that infect humans. Enhanced surveillance and further investigation are ongoing. (Funded by the National Key Research and Development Program of China and the National Major Project for Control and Prevention of Infectious Disease in China.)

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EMERGING AND REEMERGING PATHOGENS ARE GLOBAL CHALLENGES FOR public health.[1] Coronaviruses are enveloped RNA viruses that are distributed broadly among humans, other mammals, and birds and that cause respiratory, enteric, hepatic, and neurologic diseases.[2,3] Six coronavirus species are known to cause human disease.[4] Four viruses — 229E, OC43, NL63, and HKU1 — are prevalent and typically cause common cold symptoms in immunocompetent individuals.[4] The two other strains — severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) — are zoonotic in origin and have been linked to sometimes fatal illness.[5] SARS-CoV was the causal agent of the severe acute respiratory syndrome outbreaks in 2002 and 2003 in Guangdong Province, China.[6-8] MERS-CoV was the pathogen responsible for severe respiratory disease outbreaks in 2012 in the Middle East.[9] Given the high prevalence and wide distribution of coronaviruses, the large genetic diversity and frequent recombination of their genomes, and increasing human–animal interface activities, novel coronaviruses are likely to emerge periodically in humans owing to frequent cross-species infections and occasional spillover events.[5,10]

In late December 2019, several local health facilities reported clusters of patients with pneumonia of unknown cause that were epidemiologically linked to a seafood and wet animal wholesale market in Wuhan, Hubei Province, China.[11] On December 31, 2019, the Chinese Center for Disease Control and Prevention (China CDC) dispatched a rapid response team to accompany Hubei provincial and Wuhan city health authorities and to conduct an epidemiologic and etiologic investigation.

We report the results of this investigation, identifying the source of the pneumonia clusters, and describe a novel coronavirus detected in patients with pneumonia whose specimens were tested by the China CDC at an early stage of the outbreak. We also describe clinical features of the pneumonia in two of these patients.

METHODS

VIRAL DIAGNOSTIC METHODS

Four lower respiratory tract samples, including bronchoalveolar-lavage fluid, were collected from patients with pneumonia of unknown cause who were identified in Wuhan on December 21, 2019, or later and who had been present at the Huanan Seafood Market close to the time of their clinical presentation. Seven bronchoalveolar-lavage fluid specimens were collected from patients in Beijing hospitals with pneumonia of known cause to serve as control samples. Extraction of nucleic acids from clinical samples (including uninfected cultures that served as negative controls) was performed with a High Pure Viral Nucleic Acid Kit, as described by the manufacturer (Roche). Extracted nucleic acid samples were tested for viruses and bacteria by polymerase chain reaction (PCR), using the RespiFinderSmart22kit (PathoFinder BV) and the LightCycler 480 real-time PCR system, in accordance with manufacturer instructions.[12] Samples were analyzed for 22 pathogens (18 viruses and 4 bacteria) as detailed in the Supplementary Appendix. In addition, unbiased, high-throughput sequencing, described previously,[13] was used to discover microbial sequences not identifiable by the means described above. A real-time reverse transcription PCR (RT-PCR) assay was used to detect viral RNA by targeting a consensus RdRp region of pan β-CoV, as described in the Supplementary Appendix.

ISOLATION OF VIRUS

Bronchoalveolar-lavage fluid samples were collected in sterile cups to which virus transport medium was added. Samples were then centrifuged to remove cellular debris. The supernatant was inoculated on human airway epithelial cells,[14] which had been obtained from airway specimens resected from patients undergoing surgery for lung cancer and were confirmed to be special-pathogen-free by NGS.[13]

Human airway epithelial cells were expanded on plastic substrate to generate passage-1 cells and were subsequently plated at a density of 2.5×10^5 cells per well on permeable Transwell-COL (12-mm diameter) supports. Human airway epithelial cell cultures were generated in an air–liquid interface for 4 to 6 weeks to form well-differentiated, polarized cultures resembling in vivo pseudostratified mucociliary epithelium.[13]

Prior to infection, apical surfaces of the human airway epithelial cells were washed three times with phosphate-buffered saline; 150 μl of supernatant from bronchoalveolar-lavage fluid samples was inoculated onto the apical surface of the cell cultures. After a 2-hour incubation at 37°C, unbound virus was removed by washing with 500 μl of phosphate-buffered saline for 10 minutes; human airway epithelial cells were maintained in an air–liquid interface incubated at 37°C with 5% carbon dioxide. Every 48 hours, 150 μl of phosphate-buffered saline was applied to the apical surfaces of the human airway epithelial cells, and after 10 minutes of incubation at 37°C the samples were harvested. Pseudostratified mucociliary epithelium cells were maintained in this environment; apical samples were passaged in a 1:3 diluted vial stock to new cells. The cells were monitored daily with light microscopy, for cytopathic effects, and with RT-PCR, for the presence of viral nucleic acid in the supernatant. After three passages, apical samples and human airway epithelial cells were prepared for transmission electron microscopy.

TRANSMISSION ELECTRON MICROSCOPY

Supernatant from human airway epithelial cell cultures that showed cytopathic effects was collected, inactivated with 2% paraformaldehyde for at least 2 hours, and ultracentrifuged to sediment virus particles. The enriched supernatant was negatively stained on film-coated grids for examination. Human airway epithelial cells showing cytopathic effects were collected and fixed with 2% paraformaldehyde–2.5% glutaraldehyde and were then fixed with 1% osmium tetroxide dehydrated with grade ethanol embedded with PON812 resin. Sections (80 nm) were cut from resin block and stained with uranyl acetate and lead citrate, separately. The negative stained grids and ultrathin sections were observed under transmission electron microscopy.

VIRAL GENOME SEQUENCING

RNA extracted from bronchoalveolar-lavage fluid and culture supernatants was used as a template to clone and sequence the genome. We used a combination of Illumina sequencing and nanopore sequencing to characterize the virus genome. Sequence reads were assembled into contig maps (a set of overlapping DNA segments) with the use of CLC Genomics software, version 4.6.1 (CLC Bio). Specific primers were subsequently designed for PCR, and 5′- or 3′- RACE (rapid amplification of cDNA ends) was used to fill genome gaps from conventional Sanger sequencing. These PCR products were purified from gels and sequenced with a BigDye Terminator v3.1 Cycle Sequencing Kit and a 3130XL Genetic Analyzer, in accordance with the manufacturers' instructions.

Multiple-sequence alignment of the 2019-nCoV and reference sequences was performed with the use of Muscle. Phylogenetic analysis of the complete genomes was performed with RAxML (13) with 1000 bootstrap replicates and a general time-reversible model used as the nucleotide substitution model.

RESULTS

PATIENTS

Three adult patients presented with severe pneumonia and were admitted to a hospital in Wuhan on December 27, 2019. Patient 1 was a 49-year-old woman, Patient 2 was a 61-year-old man, and Patient 3 was a 32-year-old man. Clinical profiles were available for Patients 1 and 2. Patient 1 reported having no underlying chronic medical conditions but reported fever (temperature, 37°C to 38°C) and cough with chest discomfort on December 23, 2019. Four days after the onset of illness, her cough and chest discomfort worsened, but the fever was reduced; a diagnosis of pneumonia was based on computed tomographic (CT) scan. Her occupation was retailer in the seafood wholesale market. Patient 2 initially reported fever and cough on December 20, 2019; respiratory distress developed 7 days after the onset of illness and worsened over the next 2 days (see chest radiographs, Fig. 1), at which time mechanical ventilation was started. He had been a frequent visitor to the seafood wholesale market. Patients 1 and 3 recovered and were discharged from the hospital on January 16, 2020. Patient 2 died on January 9, 2020. No biopsy specimens were obtained.

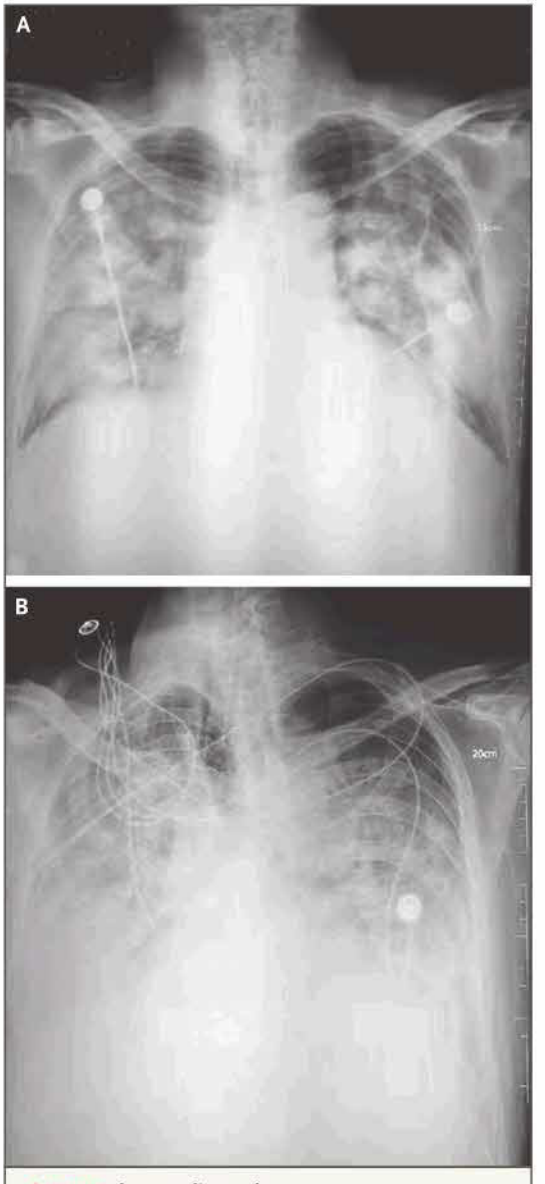

Figure 1. Chest Radiographs.

Shown are chest radiographs from Patient 2 on days 8 and 11 after the onset of illness. The trachea was intubated and mechanical ventilation instituted in the period between the acquisition of the two images. Bilateral fluffy opacities are present in both images but are increased in density, profusion, and confluence in the second image; these changes are most marked in the lower lung fields. Changes consistent with the accumulation of pleural liquid are also visible in the second image.

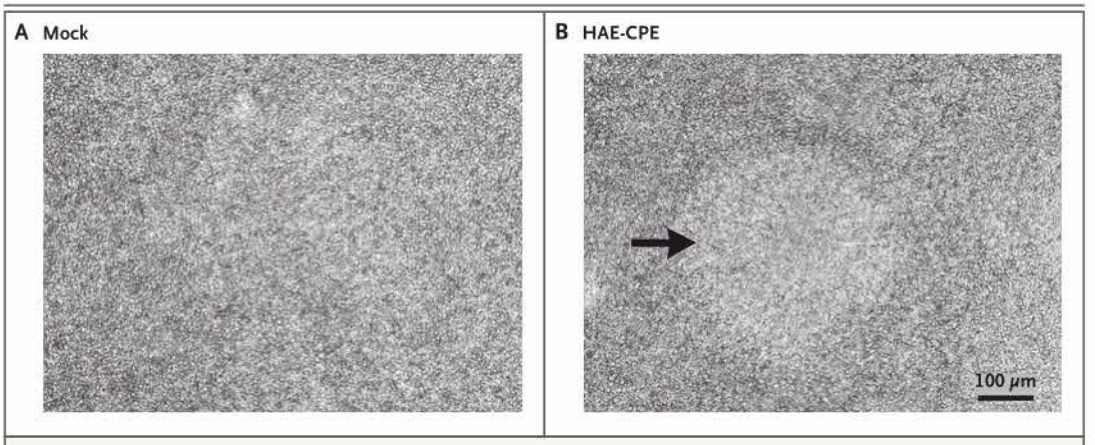

Figure 2. Cytopathic Effects in Human Airway Epithelial Cell Cultures after Inoculation with 2019-nCoV.

DETECTION AND ISOLATION OF A NOVEL CORONAVIRUS

Three bronchoalveolar-lavage samples were collected from Wuhan Jinyintan Hospital on December 30, 2019. No specific pathogens (including HCoV-229E, HCoV-NL63, HCoV-OC43, and HCoV-HKU1) were detected in clinical specimens from these patients by the RespiFinderSmart-22kit. RNA extracted from bronchoalveolar-lavage fluid from the patients was used as a template to clone and sequence a genome using a combination of Illumina sequencing and nanopore sequencing. More than 20,000 viral reads from individual specimens were obtained, and most contigs matched to the genome from lineage B of the genus betacoronavirus — showing more than 85% identity with a bat SARS-like CoV (bat-SL-CoVZC45, MG772933.1) genome published previously. Positive results were also obtained with use of a real-time RT-PCR assay for RNA targeting to a consensus RdRp region of pan β-CoV (although the cycle threshold value was higher than 34 for detected samples). Virus isolation from the clinical specimens was performed with human airway epithelial cells and Vero E6 and Huh-7 cell lines. The isolated virus was named 2019-nCoV.

To determine whether virus particles could be visualized in 2019-nCoV–infected human airway epithelial cells, mock-infected and 2019-nCoV–infected human airway epithelial cultures were examined with light microscopy daily and with transmission electron microscopy 6 days after inoculation. Cytopathic effects were observed 96 hours after inoculation on surface layers of human airway epithelial cells; a lack of cilium beating was seen with light microcopy in the center of the focus (Fig. 2). No specific cytopathic effects were observed in the Vero E6 and Huh-7 cell lines until 6 days after inoculation.

Electron micrographs of negative-stained 2019-nCoV particles were generally spherical with some pleomorphism (Fig. 3). Diameter varied from about 60 to 140 nm. Virus particles had quite distinctive spikes, about 9 to 12 nm, and gave virions the appearance of a solar corona. Extracellular free virus particles and inclusion bodies filled with virus particles in membrane-bound vesicles in cytoplasm were found in the human airway epithelial ultrathin sections. This observed morphology is consistent with the Coronaviridae family.

To further characterize the virus, de novo sequences of 2019-nCoV genome from clinical specimens (bronchoalveolar-lavage fluid) and human airway epithelial cell virus isolates were obtained by Illumina and nanopore sequencing. The novel coronavirus was identified from all three patients. Two nearly full-length coronavirus sequences were obtained from bronchoalveolar-lavage fluid (BetaCoV/Wuhan/IVDC-HB-04/2020, BetaCoV/Wuhan/IVDC-HB-05/2020|EPI_ISL_402121), and one full-length sequence was obtained from a virus isolated from a patient (BetaCoV/Wuhan/IVDC-HB-01/2020|EPI_ISL_402119). Complete genome sequences of the three novel coronaviruses were submitted to GASAID (BetaCoV/Wuhan/IVDC-HB-01/2019, accession ID: EPI_ISL_402119; BetaCoV/Wuhan/IVDC-HB-04/2020, accession ID: EPI_ISL_402120; BetaCoV/Wuhan/IVDC-HB-05/2019,

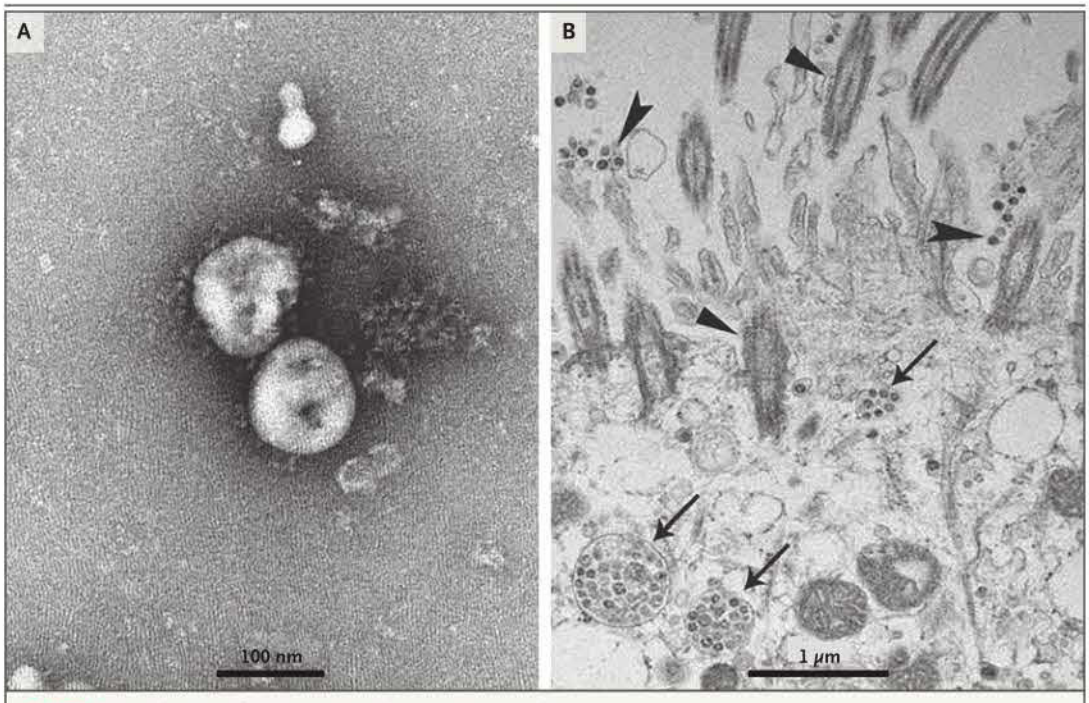

Figure 3. Visualization of 2019-nCoV with Transmission Electron Microscopy.
Negative-stained 2019-nCoV particles are shown in Panel A, and 2019-nCoV particles in the human airway epithelial cell ultrathin sections are shown in Panel B.

accession ID: EPI_ISL_402121) and have a 86.9% nucleotide sequence identity to a previously published bat SARS-like CoV (bat-SL-CoVZC45, MG772933.1) genome. The three 2019-nCoV genomes clustered together and formed an independent subclade within the sarbecovirus subgenus, which shows the typical betacoronavirus organization: a 5′ untranslated region (UTR), replicase complex (orf1ab), S gene, E gene, M gene, N gene, 3′ UTR, and several unidentified nonstructural open reading frames.

Although 2019-nCoV is similar to some betacoronaviruses detected in bats (Fig. 4), it is distinct from SARS-CoV and MERS-CoV. The three 2019-nCoV coronaviruses from Wuhan, together with two bat-derived SARS-like strains, ZC45 and ZXC21, form a distinct clade in lineage B of the subgenus sarbecovirus. SARS-CoV strains from humans and genetically similar SARS-like coronaviruses from bats collected from southwestern China formed another clade within the subgenus sarbecovirus. Since the sequence identity in conserved replicase domains (ORF 1ab) is less than 90% between 2019-nCoV and other members of betacoronavirus, the 2019-nCoV — the likely causative agent of the viral pneumonia in Wuhan — is a novel betacoronavirus belonging to the sarbecovirus subgenus of Coronaviridae family.

DISCUSSION

We report a novel CoV (2019-nCoV) that was identified in hospitalized patients in Wuhan, China, in December 2019 and January 2020. Evidence for the presence of this virus includes identification in bronchoalveolar-lavage fluid in three patients by whole-genome sequencing, direct PCR, and culture. The illness likely to have been caused by this CoV was named "novel coronavirus-infected pneumonia" (NCIP). Complete genomes were submitted to GASAID. Phylogenetic analysis revealed that 2019-nCoV falls into the genus betacoronavirus, which includes coronaviruses (SARS-CoV, bat SARS-like CoV, and others) discovered in humans, bats, and other wild animals.[15] We report isolation of the virus and the initial description of its specific cytopathic effects and morphology.

Molecular techniques have been used suc-

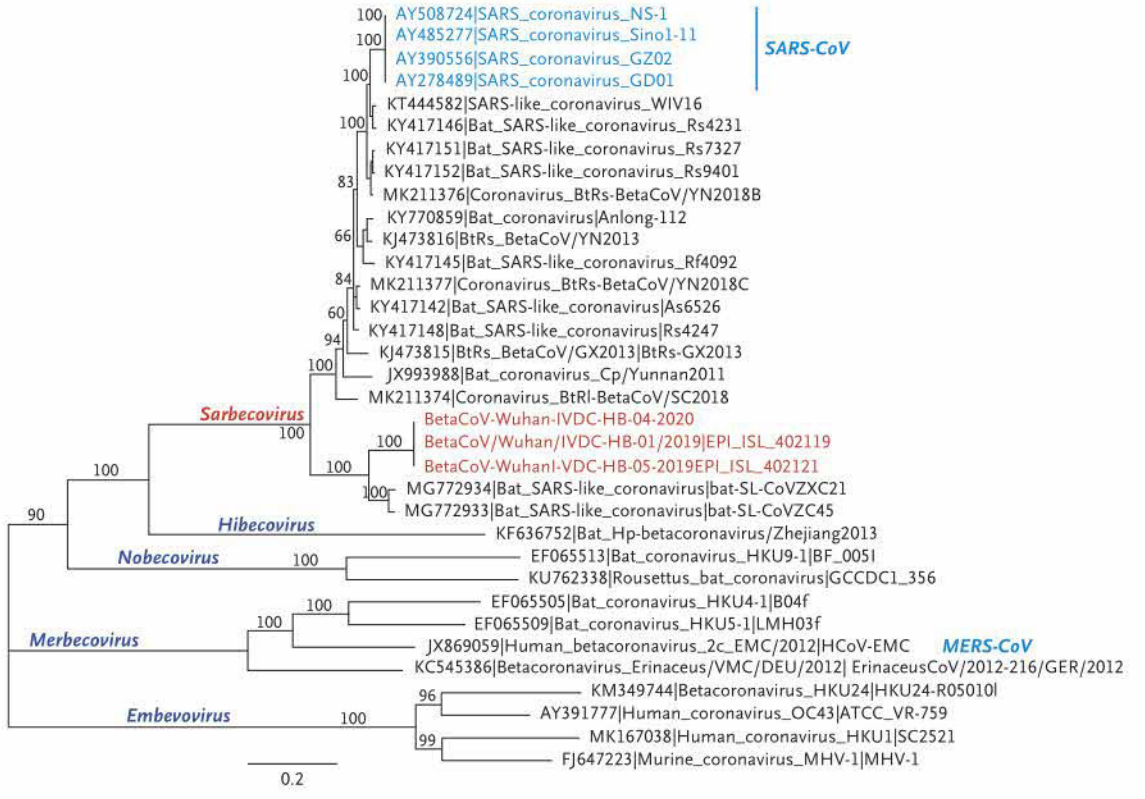

Figure 4. Phylogenetic Analysis of 2019-nCoV and Other Betacoronavirus Genomes in the Orthocoronavirinae Subfamily.

cessfully to identify infectious agents for many years. Unbiased, high-throughput sequencing is a powerful tool for the discovery of pathogens.[14,16] Next-generation sequencing and bioinformatics are changing the way we can respond to infectious disease outbreaks, improving our understanding of disease occurrence and transmission, accelerating the identification of pathogens, and promoting data sharing. We describe in this report the use of molecular techniques and unbiased DNA sequencing to discover a novel betacoronavirus that is likely to have been the cause of severe pneumonia in three patients in Wuhan, China.

Although establishing human airway epithelial cell cultures is labor intensive, they appear to be a valuable research tool for analysis of human respiratory pathogens.[14] Our study showed that initial propagation of human respiratory secretions onto human airway epithelial cell cultures, followed by transmission electron microscopy and whole genome sequencing of culture supernatant, was successfully used for visualization and detection of new human coronavirus that can possibly elude identification by traditional approaches.

Further development of accurate and rapid methods to identify unknown respiratory pathogens is still needed. On the basis of analysis of three complete genomes obtained in this study, we designed several specific and sensitive assays targeting ORF1ab, N, and E regions of the 2019-nCoV genome to detect viral RNA in clinical specimens. The primer sets and standard operating procedures have been shared with the World Health Organization and are intended for surveillance and detection of 2019-nCoV infection globally and in China. More recent data show 2019-nCoV detection in 830 persons in China.[17]

Although our study does not fulfill Koch's postulates, our analyses provide evidence implicating 2019-nCoV in the Wuhan outbreak. Additional evidence to confirm the etiologic sig-

nificance of 2019-nCoV in the Wuhan outbreak include identification of a 2019-nCoV antigen in the lung tissue of patients by immunohistochemical analysis, detection of IgM and IgG antiviral antibodies in the serum samples from a patient at two time points to demonstrate seroconversion, and animal (monkey) experiments to provide evidence of pathogenicity. Of critical importance are epidemiologic investigations to characterize transmission modes, reproduction interval, and clinical spectrum resulting from infection to inform and refine strategies that can prevent, control, and stop the spread of 2019-nCoV.

This work was supported by grants from the National Key Research and Development Program of China (2016YFD0500301) and the National Major Project for Control and Prevention of Infectious Disease in China (2018ZX10101002).

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

We thank Dr. Zhongjie Li, Dr. Guangxue He, Dr. Lance Rodewald, Yu Li, Fei Ye, Li Zhao, Weimin Zhou, Jun Liu, Yao Meng, Huijuan Wang, and many staff members at the China CDC for their contributions and assistance in this preparation and submission of an earlier version of the manuscript.

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From:	Tamin, Azaibi (CDC/DDID/NCIRD/DVD)
To:	Lindstrom, Stephen (CDC/DDID/NCIRD/DVD); Tong, Suxiang (Sue) (CDC/DDID/NCIRD/DVD); Thornburg, Natalie (CDC/DDID/NCIRD/DVD)
Cc:	Harcourt, Jennifer (CDC/DDID/NCIRD/DVD); Kamili, Shifaq (CDC/DDID/NCIRD/DVD) (CTR); Sakthivel, Senthil Kumar K. (CDC/DDID/NCIRD/DVD) (CTR)
Subject:	RE: Scope pics of potential 2019 N-CoV from the 1st US case
Date:	Saturday, January 25, 2020 2:37:18 PM
Attachments:	2019 NCoV_scope pics of potential isolates from 1st US case_V2.pdf

Hi Steve,
I placed 29 samples of potential 2019 N-CoV isolates in AVL lysis buffer (350 uls AVL + 50 uls lysates in a plastic box labeled 'AT samples 1/24/20" - in the bottom shelf in a larger open box in -70C freezer #5 in the LER.

The tubes are labeled 'A1 to A12'; 'B2 to B10' (NP645); 'E1 to E4'; and 'F2 to F5' (OP646).

If your team cant test all of them, the priority is to test on the tubes with the highest dilutions – **A12, B10, E4** and **F5**.

Thank you,
AT

From: Lindstrom, Stephen (CDC/DDID/NCIRD/DVD) <sql5@cdc.gov>
Sent: Saturday, January 25, 2020 1:41 PM
To: Tong, Suxiang (Sue) (CDC/DDID/NCIRD/DVD) <sot1@cdc.gov>; Tamin, Azaibi (CDC/DDID/NCIRD/DVD) <axt4@cdc.gov>; Thornburg, Natalie (CDC/DDID/NCIRD/DVD) <nax3@cdc.gov>
Cc: Harcourt, Jennifer (CDC/DDID/NCIRD/DVD) <zaq6@cdc.gov>
Subject: RE: Scope pics of potential 2019 N-CoV from the 1st US case

Very nice unhappy cells!

From: Tong, Suxiang (Sue) (CDC/DDID/NCIRD/DVD) <sot1@cdc.gov>
Sent: Saturday, January 25, 2020 12:24 PM
To: Tamin, Azaibi (CDC/DDID/NCIRD/DVD) <axt4@cdc.gov>; Thornburg, Natalie (CDC/DDID/NCIRD/DVD) <nax3@cdc.gov>
Cc: Lindstrom, Stephen (CDC/DDID/NCIRD/DVD) <sql5@cdc.gov>; Harcourt, Jennifer (CDC/DDID/NCIRD/DVD) <zaq6@cdc.gov>
Subject: RE: Scope pics of potential 2019 N-CoV from the 1st US case

Cheers!!

From: Tamin, Azaibi (CDC/DDID/NCIRD/DVD) <axt4@cdc.gov>
Sent: Saturday, January 25, 2020 12:13 PM
To: Thornburg, Natalie (CDC/DDID/NCIRD/DVD) <nax3@cdc.gov>
Cc: Lindstrom, Stephen (CDC/DDID/NCIRD/DVD) <sql5@cdc.gov>; Tong, Suxiang (Sue)

Scope pictures of potential 2019 N-CoV isolates from the 1st US case (10X/passage 1/2 days post infection)

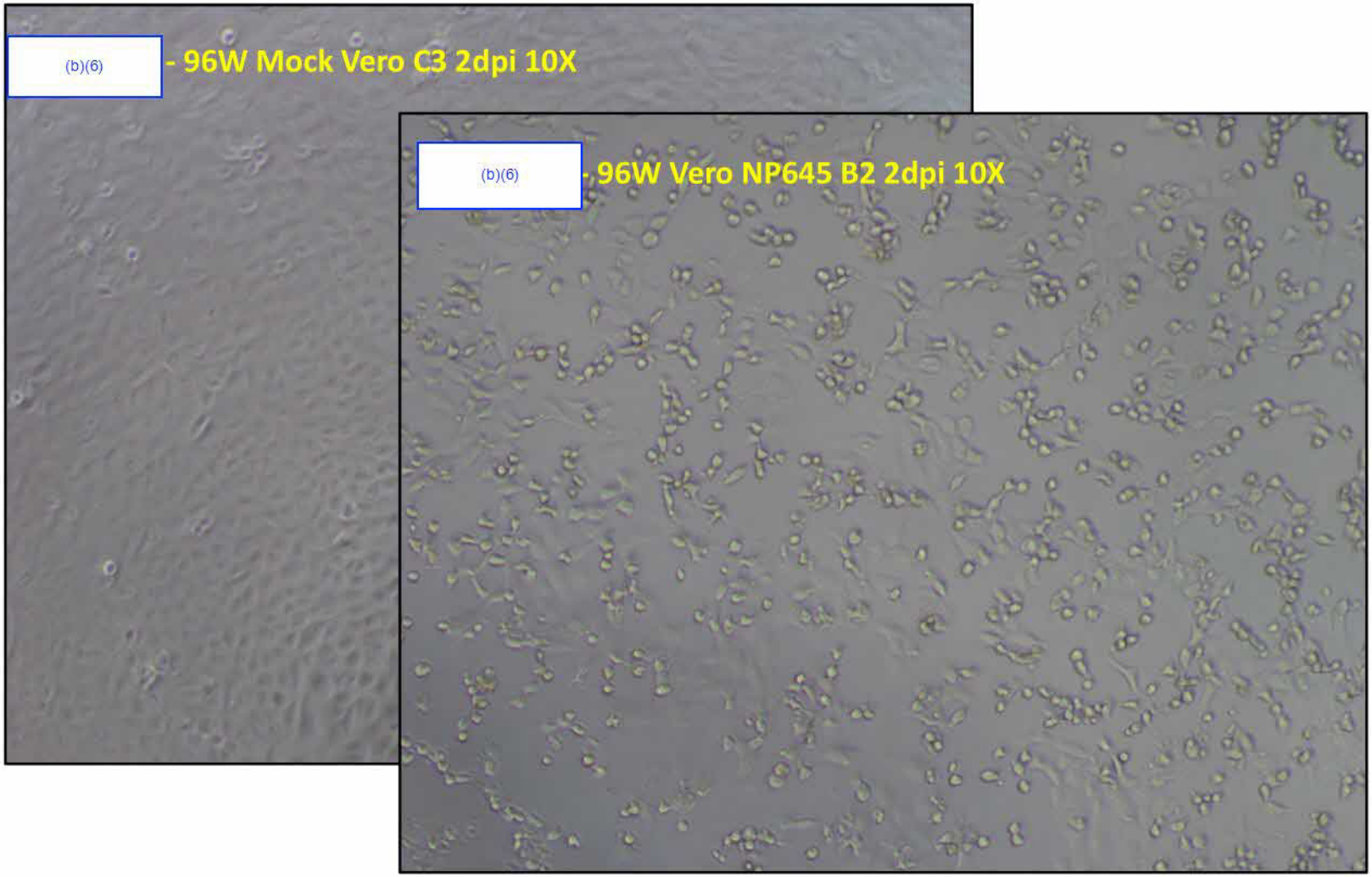
Brief method:

Use serial dilution in a 96 wells plate format;

Vero cells suspension [~2.5 x 10^4 cells] in DMEM/2X PS/Fungizone

(b)(6) - 96W Mock Vero C3 2dpi 10X

(b)(6) - 96W Vero NP645 B3 2dpi 10X


(b)(6)
- 96W Mock Vero C3 2dpi 10X
(b)(6)
- 96W Vero NP645 B2 2dpi 10X


(b)(6)
96W Mock Vero C3 2dpi 10X
(b)(6)
- 96W Vero NP645 A1 2dpi 10X
(b)(6)
96W Vero NP645 A2 2dpi 10X


(b)(6)
96W Mock Vero C2 2dpi 10X
(b)(6)
96W Vero NP645 A3 2dpi 10X
(b)(6)
96W Vero NP645 A4 2dpi 10X


(b)(6)
96W Mock Vero C2 2dpi 10X
(b)(6)
96W Vero NP645 A5 2dpi 10X

(CDC/DDID/NCIRD/DVD) <sot1@cdc.gov>; Harcourt, Jennifer (CDC/DDID/NCIRD/DVD) <zaq6@cdc.gov>
Subject: Scope pics of potential 2019 N-CoV from the 1st US case

Hi gang,
Hope some of these 7 lysates that show CPE are caused by the 2019 N-CoV

Cheers,
AT

Azaibi Tamin, Ph.D.
Research Microbiologist,
Respiratory Viruses Imunology Team, Respiratory Viruses Branch,
Division of Viral Diseases, National Center for Immunization and Respiratory Disease,
Centers for Disease Control and Prevention, Atlanta GA 30333, USA
Email: atamin@cdc.gov Tel: (404) 639 1302 Cell: (b)(6)

From: Murray, Janna' R. (CDC/DDID/NCIRD/DVD) (CTR)
Sent: Wed, 29 Jan 2020 21:48:49 +0000
To: Tamin, Azaibi (CDC/DDID/NCIRD/DVD); Thornburg, Natalie (CDC/DDID/NCIRD/DVD)
Cc: Lindstrom, Stephen (CDC/DDID/NCIRD/DVD)
Subject: Real-time PCR results for virus samples submitted
Attachments: 01-29-2020 Virus Testing for Natalie's Team.xlsx

Hi Azaibi and Natalie,

Attached are the Real-time PCR results for the 4 viruses you submitted for testing. Each sample was tested in triplicate so there are 3 Ct values for each. The samples are named according to what was written on the tube. Let me know if you have any questions.

Regards,
Janná

Janná Murray, MPH
Microbiologist (Eagle Contractor)
Respiratory Viruses Diagnostic Laboratory
Respiratory Viruses Branch
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1600 Clifton Road NE, MS-G04 Atlanta, GA 30329-4027
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Results Reporting Worksheet

Lab ID:	CDC/RVB-DX	**Instrument Manufacturer**	ABI
Operator:	Janna Murray	**Instrument Model**	7500Fast
Run Date:	1/0/1900	**Kit**	**TaqPath 1-Step RT-PCR Master Mix**
File Name:	01-29-2020 Virus Testing for Natalie's Team	**Kit Lot No**	
		Extraction Date and Batch #:	
		Master Mix Batch #	

Thermal Profile (cycling parameters)

Stage	Temperature	Duration	#cycles	
cycle 1	25	2 min	1	
cycle2	50	15 min	1	
cycle 3	95	2 min	1	
cycle4	95	3 sec	45	
	55	30 sec	collect	

		Man	**Cycle**	**3**
SDS Software version		**1.4.1**	**Cycle**	**15**

***enter sample names into instrument run setup software exactly as it appears on the master mix sheet*

Virus_Name	19-nCoV_N1_C	2019-nCoV_N1_Ct2	2019-nCoV_N1_Ct3	2019-nCoV_N2_Ct2	2019-nCoV_N2_Ct4	2019-nCoV_N2_Ct5	2019-nCoV_N2_Ct6	2019-nCoV_N3_Ct3	2019-nCoV_N3_Ct7	2019-nCoV_N3_Ct8	2019-nCoV_N3_Ct9
A12	16.04	16.18	16.14								
B10	16.19	16.2	16.16								
E4	17.11	17.08									
NTC	0.00										
A12					15.92	16.05	16.07				
B10					16.07	16.1	16.05				
E4					17.04	17.07					
NTC				0							
A12									16.2	16.22	16.31
B10									16.19	16.22	16.2
E4									17.21	17.28	
NTC								0			
F6	17.27	17.36	17.3								
F6					17.2	17.1	17.26				
F6									17.33	17.21	17.26
E4			17.1				16.98				17.32
PC	25.70			27.06				26.37			
Analysis Threshold Setting											
NTC Valid		**Yes**		**PC Ct < 37**	**Yes**			**Valid Run**			**Yes**

Comments:

Supervisor Reviewed Signature / Date

X

Supervisor signature

X

Brett Whitaker
Tester Signature

Extraction Tech:		**Extraction Kit Manufacturer:**	**QIAGEN**
Extraction Date:		**Extraction Kit Used:**	**EZ1 DSP Virus Kit/Buffer AVL**
Lab I.D. :	CDC/Influenza	**Extraction Kit Lot # :**	
		Extraction Kit Exp Date :	

Extraction Batch :

	Lysis Buffer vol.	Specimen vol.	Elution vol.	**Sample Passed?**		Lysis Buffer vol.	Specimen vol.	Elution vol.	**Sample Passed?**
Sample ID					Sample ID				
Sample ID					Sample ID				
Sample ID					Sample ID				
Sample ID					Sample ID				
Sample ID					Sample ID				
Sample ID					Sample ID				
Sample ID					Sample ID				
Sample ID					Sample ID				
Sample ID					Sample ID				

Comments:

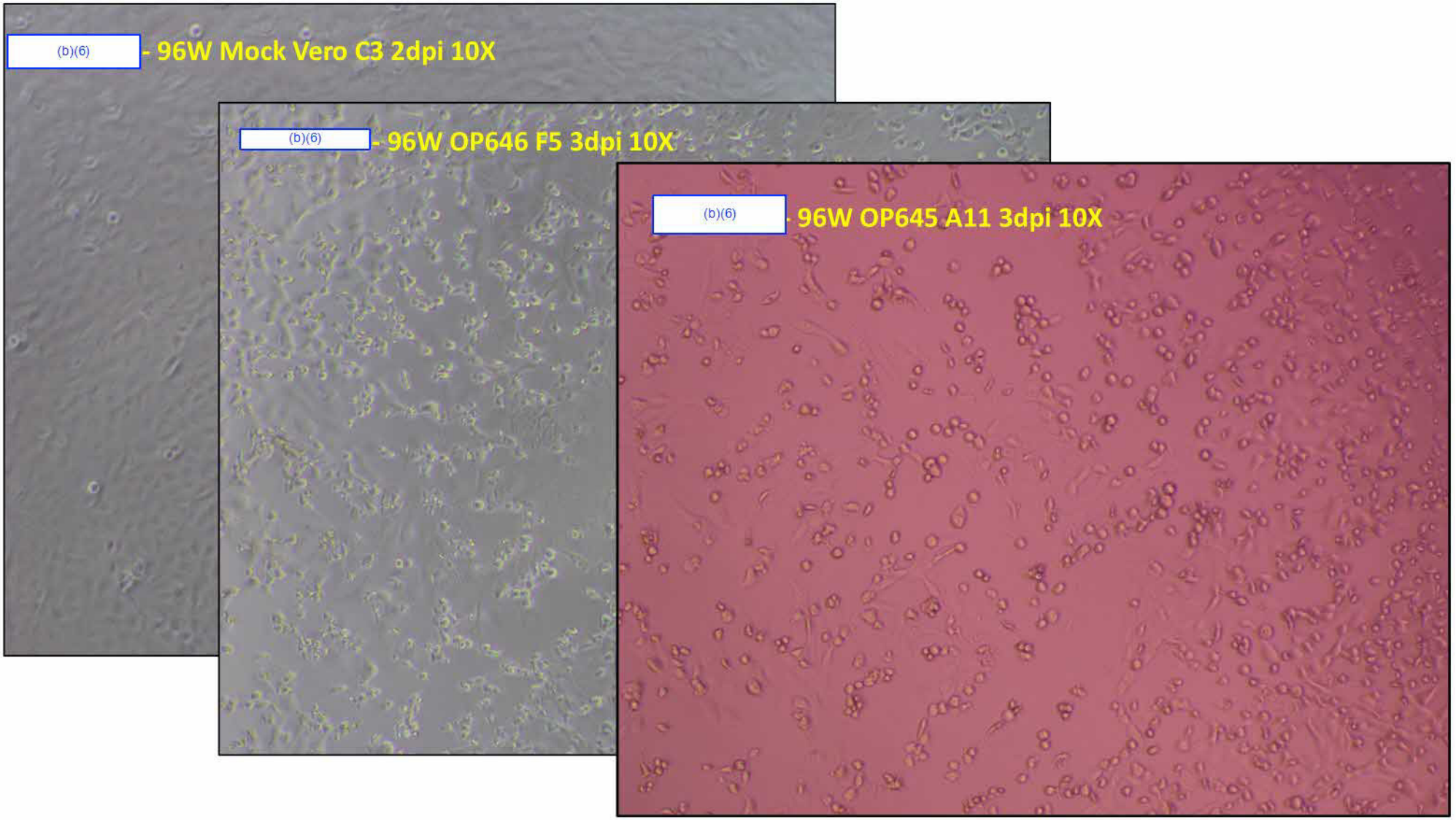
Review By: _____ Date: _____

From: Tamin, Azaibi (CDC/DDID/NCIRD/DVD)
Sent: Mon, 27 Jan 2020 18:42:39 +0000
To: Tong, Suxiang (Sue) (CDC/DDID/NCIRD/DVD); Queen, Krista (CDC/DDID/NCIRD/DVD)
Cc: Thornburg, Natalie (CDC/DDID/NCIRD/DVD); Harcourt, Jennifer (CDC/DDID/NCIRD/DVD)
Subject: RT-PCR confirmation for 2 potential isolates of 2019 N CoV
Attachments: 2019 NCoV_2 potential isolates from the 1st US case.pdf

Hi Sue and Krista,
Jennifer Harcourt have passed to you 2 lysates (passage 1) from the case #1 US case, in AVL lysis buffer (350 uls AVL + 50 uls virus lysates) for molecular confirmation. Attached are their scope pics at 3 days post infection. Thank you for your time and help.

Cheers,
AT

Azaibi Tamin, Ph.D.
Research Microbiologist,
Respiratory Viruses Imunology Team, Respiratory Viruses Branch,
Division of Viral Diseases, National Center for Immunization and Respiratory Disease,
Centers for Disease Control and Prevention, Atlanta GA 30333, USA
Email: atamin@cdc.gov Tel: (404) 639 1302 Cell: (b)(6)


(b)(6)
- 96W Mock Vero C3 2dpi 10X
(b)(6)
- 96W OP646 F5 3dpi 10X
(b)(6)
- 96W OP645 A11 3dpi 10X

Gmail

Your CDC FOIA Request #21-01704-FOIA

[redacted] Wed, Mar 30, 2022 at 7:17 AM

To: PThomas3@cdc.gov

Greetings,

You did not provide any information related to the Control Groups requested. You provided irrelevant communication between people, a paper that never isolated and purified SARS-COV-2, and a spreadsheet that has nothing to do with control information.

Here is the request Again:

Control Group Information:

- Did the scientist for this paper use control groups?
- If so, did the control groups use the same formulations of cell culture mixtures as the experimental groups sans the sample containing the alleged viruses?
- For instance, the experimental groups contained the following contents at the specified volumes:
  - viral transport medium
  - 2× penicillin/streptomycin
  - 2× antibiotics/antimycotics
  - 2× amphotericin B
  - 10% fetal bovine serum
- Did the control groups use the same volume and type of nutrient solution?
  - For instance, Dulbecco minimal essential medium (DMEM)

In summary, if control groups were used, please list details of the control groups.

Please only provide control information for the paper listed. If you have no controls, then simply write you have no control information (because your experiment is unscientific).

Thank you

[Quoted text hidden]

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