



2020-Our file: PHAC-A-2020-000110 / TTL

2020-12-07

Christine Massey



Dear Christine Massey:

This is in response to your request made under the *Access to Information Act* (the Act) for the following information:

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

Clarification:

Date range of request is January 1, 2020 until June 15, 2020

Enclosed please find records responsive to your request. You will note that portions of the records are withheld from disclosure pursuant to sections 19 and 20 of the Act. For ease of reference, a copy of the Act may be found at <https://laws-lois.justice.gc.ca/eng/acts/a-1/> which provides a description of the redaction(s) applied.

Should you have any questions or concerns about the processing of your request please do not hesitate to contact Tammy Turpin-Loyer, the analyst responsible for this file, by email at tammy.turpin-loyer@canada.ca with reference to our file number cited above.

Please be advised that you are entitled to complain to the Office of the Information Commissioner of Canada concerning the processing of your request within 60 days of the receipt of this notice. In the event you decide to avail yourself of this right, your notice of complaint can be made online at: <https://www.oic-ci.gc.ca/en/submitting-complaint> or by mail to:

Office of the Information Commissioner of Canada
30 Victoria Street
Gatineau, Quebec K1A 1H3

Yours sincerely,



Digitally signed by Mathews, Curtis
DN: C=CA, O=GC, OU=HC-SC,
CN="Mathews, Curtis"
Date: 2020-12-07 14:18:56

Curtis Mathews
Manager
Access to Information and Privacy Division

Enclosure: Release package



Public Health
Agency of Canada

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Li, Yan (PHAC/ASPC)

From: [REDACTED]
Sent: 2020-02-20 1:54 PM
To: Li, Yan (PHAC/ASPC)
Subject: Re: Shipping delayed
Attachments: 20200220 - 145354 Scan.pdf; Fig1.pptx

Hi Yan,

The package has been picked up by FedEx. The tracking number is 808209522519.

For p.2 I don't know. We still have not been able to get to the PCR . I would think it should be either the same titer as p.1 or higher. So 15ul to 30ul in a T75 should be enough. And we just setup plates today to do the titration.

To make p.2 I had 1ug/ml of TPCK trypsin in the media (DMEM, 2% FBS, P/S) during the virus absorption, removed the inoculum after 1hr and replaced with fresh media containing 2% FBS, penn/strep and 1ug/ml TPCK trypsin (so the same media as the adsorption). You are correct. We did NOT perform centrifugation or high trypsin when we used p.1 to make p.2. Just a normal infection procedure (except with the addition of 1ug/ml TCPK trypsin).

I attached a figure from our grant. The CPE and RT-PCR on p.1 is in there. Obvious differences between the sensitivity of the primers (although, we used one concentration of primer/probes, and not the suggested concentrations that was suggested).

Hope this helps. And I hope everything is fine with the samples we sent. We still need to do some verification but I figured since p.1 looks like SARS-CoV-2 (because of the PCR) and CPE was on-time and as expected for p.2 that we would ship to you now instead of waiting to make sure everything is as expected. Certainly we will let you know if we come across any results that are not consistent with what we expect.

Thanks

[REDACTED]

From: "Li, Yan (PHAC/ASPC)" <yan.li@canada.ca>
Date: Thursday, February 20, 2020 at 12:53 PM
To: [REDACTED]
Subject: RE: Shipping delayed

CAUTION: This email originated from outside [REDACTED] Do not click links or open attachments unless you recognize the sender and know the content is safe. If in doubt, please forward suspicious emails to phishing [REDACTED]

Hi [REDACTED]

ATIA - 19(1)

I forgot to mention that we would need the waybill number in case there is some problem with the delivery.

As you indicated that you will send 2x 1 ml p2 and 100 ul P1. How much of P2 would you recommend to use for infecting a T75 flask? We also like to confirm that concentration of trypsin you are using now when you pass the virus (16 ug/ml or 1ug/ml). As we understand, the centrifugation will not be required to pass the virus. By the way, do you know the CT value for P1 and P2 virus?

Thank you so much for your help,

Yan

From: [REDACTED]

Sent: 2020-02-19 7:21 PM

To: Li, Yan (PHAC/ASPC) <yan.li@canada.ca>

Subject: Shipping delayed

Hi Yan,

Despite my pleading, FedEx refused to pick up today as we apparently missed their cut-off. I apologise and we have it arranged to be picked up tomorrow.

Thanks,

Is(Are) exempted and/or excluded pursuant to section(s)
est(sont) exemptée(s) et/ou exclus en vertu de(s)(l')article(s)

20(1)(b)

Subject to this section, the head of a government institution shall refuse to disclose any record requested under this Act that contains (b) financial, commercial, scientific or technical information that is confidential information supplied to a

Le responsable d'une institution fédérale est tenu, sous réserve des autres dispositions du présent article, de refuser la communication de documents contenant : b) des renseignements financiers, commerciaux, scientifiques ou techniques fournis à

Predicting infectious SARS-CoV-2 from diagnostic samples

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Summary: Respiratory samples from COVID-19 patients with ≥ 8 days of symptoms and a SARS-CoV-2 E gene RT-PCR Ct value ≥ 24 may predict lack of infectivity of those patients in a clinical and community context.

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Abstract

Background: RT-PCR has become the primary method to diagnose viral diseases, including SARS-CoV-2. RT-PCR detects RNA, not infectious virus, thus its ability to determine duration of infectivity of patients is limited. Infectivity is a critical determinant in informing public health guidelines/interventions. Our goal was to determine the relationship between E gene SARS-CoV-2 RT-PCR cycle threshold (Ct) values from respiratory samples, symptom onset to test (STT) and infectivity in cell culture.

Methods: In this retrospective cross-sectional study, we took SARS-CoV-2 RT-PCR confirmed positive samples and determined their ability to infect Vero cell lines.

Results: Ninety RT-PCR SARS-CoV-2 positive samples were incubated on Vero cells. Twenty-six samples (28.9%) demonstrated viral growth. Median TCID₅₀/ml was 1780 (282-8511). There was no growth in samples with a Ct > 24 or STT > 8 days. Multivariate logistic regression using positive viral culture as a binary predictor variable, STT and Ct demonstrated an odds ratio for positive viral culture of 0.64 (95% CI 0.49-0.84, p<0.001) for every one unit increase in Ct. Area under the receiver operating characteristic curve for Ct vs. positive culture was OR 0.91 (95% CI 0.85-0.97, p<0.001), with 97% specificity obtained at a Ct of >24.

Conclusions: SARS-CoV-2 Vero cell infectivity was only observed for RT-PCR Ct < 24 and STT < 8 days. Infectivity of patients with Ct >24 and duration of symptoms >8 days may be low. This information can inform public health policy and guide clinical, infection control and occupational health decisions. Further studies of larger size are needed.

Keywords: SARS-COV-2, COVID-19, RT-PCR, infectivity, public health

Introduction

The emergence of SARS-CoV-2, the causative agent of COVID-19, represents a public health emergency of historic proportion. The global containment efforts have had broad societal and economic impacts. Policy decisions to relax public health measures will require a better understanding of duration of infectivity. This information will also impact infection control practices and occupational health.

To date, the diagnosis of COVID-19 has relied on the detection of SARS-CoV-2 through molecular detection. While this method is both rapid and highly sensitive, there are important limitations. Several studies describe the persistence of SARS-CoV-2 RNA within different body sites (1,2). It is known from other viruses that viral RNA can persist beyond infectivity (3,4). As a result, demonstration of *in vitro* infectiousness on cell lines is a more informative surrogate of viral transmission. The ability of viral culture to inform infectivity is an important aspect of diagnostics but its use is hampered by its difficult and labour-intensive nature. This is further complicated by the need for containment level 3 facilities in the case of SARS-CoV-2. In a recent cohort study of nine patients, no virus could be recovered beyond 7 days post symptom onset (1). This important study is limited by the small number of patients examined and the fact that all nine cases are linked, therefore the data may represent a unique viral subpopulation. Here we add to the existing body of literature by presenting viral culture results on a larger cross-sectional group of patients, compared to PCR data and time of symptom onset.

Methods

SARS-CoV-2 RT PCR cycle threshold values and symptom onset to test

All samples in this study were obtained to support routine care and surveillance of the public health response in the province of Manitoba, Canada. All suspect COVID-19 cases had SARS-CoV-2 RT-PCR performed on nasopharyngeal (NP) or endotracheal (ETT) samples at Cadham Provincial Laboratory (CPL), the public health laboratory.

NP swabs and ETT specimens in viral transport media were stored at 4°C for 24-72 hours until they were tested for the presence of SARS-CoV-2 RNA using real-time RT-PCR targeting a 122nt portion of the Sarbecovirus envelope gene (E gene) (5). Fifty-five microliters of RNA were extracted from 200 µL of a respiratory specimen using the Ambion AM1836 RNA kit (Thermofisher) paired with the Kingfisher Flex instrument (Thermofisher). The 20 µL reactions, comprised of Taqman Fast Virus One-step master mix and 5 µL of RNA, were cycled for 5 min@ 50°C, 20 sec@ 95°C followed by 40 cycles of 5 sec@ 95°C and 30 sec @ 58°C on a Biorad CFX96 thermal cycler. RT-PCR results were analyzed with the CFX manager software (version 3.1).

Through public health and epidemiology/surveillance and laboratory records, date of symptom onset was determined. Time from symptom onset to RT-PCR, or symptoms to test (STT), was calculated based on laboratory records. For all positive samples, the cycle threshold (Ct) was obtained. The study was performed in accordance with protocol HS23906 (H2020:211), approved by the University of Manitoba Research Ethics Board.

Tissue Culture Infectious Dose 50% (TCID50) Assay

Samples were stored at -80°C for between 2 to 4 weeks before being processed for culture. Viral titers of patient samples were determined through TCID50 assays inside a biocontainment level 4 laboratory (BSL4). Briefly, Vero cells (ATCC: CCL-81), maintained in Modified Eagles Medium (MEM) supplemented with 5% Fetal Bovine Serum (FBS), 1% penicillin/ streptomycin (P/S), 0.5 µg/mL Amphotericin B and 1% L-glutamine, were seeded into 96 well plates (Thermo Scientific, 167008) at 70% confluency. Using dilution blocks, patient samples were serially diluted 10-fold from 10⁻¹ to 10⁻⁸ in MEM supplemented with 2% FBS, 1% Penicillin/Streptomycin, 0.5 µg/mL Amphotericin B and 1% L-glutamine. Dilutions were placed onto the Vero cells in triplicate and incubated at 37°C with 5% CO2 for 96 hours. Following incubation of 4 days, cytopathic effect (CPE) was evaluated under a microscope and recorded. TCID50 and TCID50/mL were calculated using the Reed and Muench method previously described (6)

Statistical Methods

Data are presented as mean ± standard deviation for normally distributed data and as median [Interquartile range] for non-normally distributed data. P values are reported as two tailed. All statistical analysis was performed with Stata V14.2 (College Station, Texas, USA). Between group comparisons were performed using a Students t test or Mann-Whitney test. Normality was assessed using the Kolmogorov-Smirnov test, and logistic regression was performed with robust standard errors.

Results

A total of 90 samples were analyzed. Median age of the patients sampled was 45 (30-59). Forty nine percent of our samples were from males. SARS-CoV-2 was successfully cultivated from 26 (28.9%) of the samples. The samples included in this study included those positive for SARS-CoV-2 by RT-PCR from day of symptom onset (Day 0) up to 21 days post symptom onset. Within this range of samples, positive cultures were only observed up to day 8 post symptom onset (Figure 1). Median Ct count of all samples was 23 (IQR 17-32). The median TCID₅₀/ml was 1780 (282-8511). Positive culture samples had a significantly lower Ct when compared to culture negative samples (17 [16-18] vs 27 [22-33], $p < 0.001$, Figure 2). Symptom to test time was also significantly lower in culture positive vs. culture negative samples (3 [2-4] vs. 7 [4-11], $p < 0.001$, Figure 2).

Multivariate logistic regression using positive culture as a predictor variable (binary result) and STT, age and gender as independent variables showed Ct as being significant (OR 0.64 95% CI 0.49-0.84, $p < 0.001$). This implies that for every one unit increase in Ct, the odds of a positive culture decreased by 32%. Increasing symptom to test time was also significantly associated with a negative culture (OR 0.63, 95% CI 0.42-0.94, $p = 0.025$). For every one day increase in STT, the odds ratio of being culture positive was decreased by 37%. Receiver operating characteristic curves constructed using Ct vs. positive culture showed an area of 0.91 (95% CI 0.85-0.97, $p < 0.001$) with 97% specificity obtained at a Ct of greater than 24. Similarly, STT vs. positive culture showed an area of 0.81 (95% CI 0.0.73-0.90, $p < .001$), with 96% specificity at > 8 days. The probability of successfully cultivating SARS-CoV-2 on Vero cell culture compared to STT is demonstrated in Figure 3. The probability of obtaining a positive viral culture peaked on day 3 and decreased from that point.

Discussion

PCR and other nucleic amplification (NA) strategies have surpassed viral culture as the gold standard viral diagnostic, because of their wider application, higher sensitivity, rapid performance, and ability for field deployment. A major drawback to PCR and other diagnostic approaches (including other NA, serology, antigen detection) is that they all fail to determine virus infectivity: PCR sensitivity is excellent but specificity for detecting replicative virus is poor (13). Our study utilized a cross-sectional approach to correlate COVID-19 symptom onset to specimen collection with SARS-CoV-2 E gene RT-PCR and virus viability as determined by cell culture.

These results demonstrate that infectivity (as defined by growth in cell culture), is significantly reduced when RT-PCR Ct values are greater than 24. For every 1 unit increase in Ct, the odds ratio for infectivity decreased by 32%. The high specificity of Ct and STT suggests that Ct values greater than 24, along with duration of symptoms greater than 8 days may be used in combination to determine duration of infectivity in patients. Positive cell culture results in our study were most likely between days one and five. This finding is consistent with existing literature (1,2).

This study is the first to report a large enough data set that demonstrates a link between *in vitro* viral growth, Ct value and STT.

These results have implications for clinical care, infection prevention and control and public health.

These data can be used to efficiently target case finding efforts by better defining the period of maximal transmission risk. This will be of particular importance in the maintenance phase of the response, where case finding efforts to rapidly interrupt chains of transmission will be essential.

Isolation of COVID-19 cases in the community is typically recommended for at least ten days after symptom onset. Our data supports this approach. Jurisdictions across Canada and the US are recommending a variety of strategies to discontinue isolation of hospitalized COVID-19 cases (7-12).

Clinical criteria including 14 days from symptom onset or 72 hours symptom free (whichever is

longer) are being used in some while other jurisdictions are using two negative NP RT-PCR results 48 hours apart after 14 days of symptoms. Our data supports the former approach since RT-PCR positivity persists significantly beyond infectivity; the alternative approach may lead to unnecessary isolation, and use of PPE and testing resources. The qualitative reporting of results of SARS-CoV-2 RT-PCR as positive or negative is sufficient for diagnosis but may be supplemented by Ct, a semi-quantitative value, as well as time of symptom onset to guide infection control, public health and occupational health decisions.

Our study has important limitations. First, our study utilized a single SARS-CoV-2 gene target (E gene). Though other gene targets may offer greater specificity, SARS-CoV-2 E-gene is more consistently used in both laboratory-developed tests (LDT) and commercial assays. The testing criteria in Manitoba had sufficient pre-test probability to make the likelihood of a false positive remote. In addition, the first 71 of 90 samples were confirmed using the described protocol with CDC N1-gene target (14). Second target confirmation was discontinued at that time based on being satisfied with testing criteria and assay sensitivity to accurately identify true COVID-19 cases. Reagent supply also played a role. Second, the recall bias of symptom onset is possible, but this likely would have been equally distributed between those who were culture positive and negative. Third, the infectivity of certain individual cases and the accuracy of our culture assay may have unique variations. Though some individuals in our cross-sectional study would be considered immunocompromised, patients with these conditions could have prolonged shedding of infective SARS-CoV-2 and may not be fully represented here. Few children have been diagnosed with COVID-19 in our province (Median age of positive PCR = 45 [30-59]). With other respiratory viruses, children may have prolonged shedding. Finally, our patient numbers remain small and larger studies are needed to establish Ct criteria that reliably correlates with loss of infectivity and that utilize additional SARS-CoV-2 gene targets.

In conclusion, the SARS-CoV-2/COVID-19 pandemic represents a dynamic situation where decisions and policy must be guided by evidence. Our study showed no positive viral cultures with a Ct greater than 24 or STT greater than 8 days. The odds of a positive culture were decreased by 32% for each unit increase in Ct. This data, if confirmed, may help guide isolation, contact tracing, and testing guidelines.

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Acknowledgments

This work was supported by the collaborative efforts in the public health response to the SARS-CoV-2/COVID-19 pandemic by Manitoba Health and Cadham Provincial Laboratory (CPL) and the Public Health Agency of Canada and the National Microbiology Laboratory. A special acknowledgement to the Medical Laboratory Technologists in the Virus Detection Section of CPL. We would be blind without you.

Potential conflicts of interest: The authors have no conflicts to report.

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<https://www.cdc.gov/coronavirus/2019-ncov/lab/rt-pcr-panel-primer-probes.html>.

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Figure Legends:

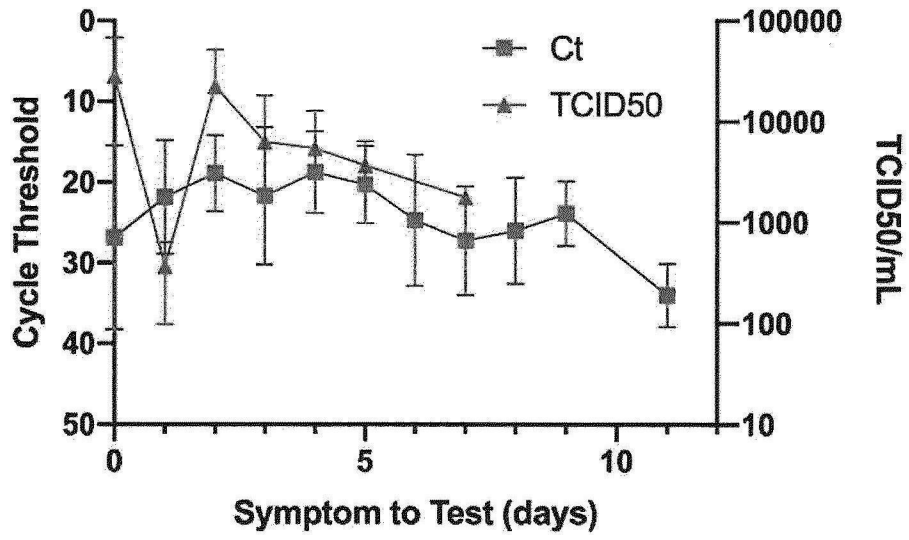
Figure 1: SARS-CoV-2 viral dynamics as expressed by E gene RT-PCR Cycle threshold (Ct) value and cell culture TCID₅₀/mL, over time (days). Squares represent Ct values while triangles reflect TCID₅₀.

Figure 2: SARS-CoV-2 E gene RT-PCR Cycle Threshold (Ct) values and symptom to test time (STT) in samples that were culture positive (Ct +, STT +), or negative (Ct -, STT -). Positive SARS-CoV-2 culture samples had a significantly lower Ct when compared to culture negative samples (17 [16-18] vs 27 [22-33], $p < 0.001$). Symptom to test time was also significantly lower in culture positive vs. culture negative samples (3 [2-4] vs. 7 [4-11], $p < 0.001$).

Figure 3: Comparison of symptom onset to test (days) to the probability of successful cultivation on Vero cells and SARS-CoV-2 E gene RT-PCR Cycle threshold (Ct) value. Ct values are represented by the line graph with circles. Probability of SARS-CoV-2 culture is shown by the bar graph.

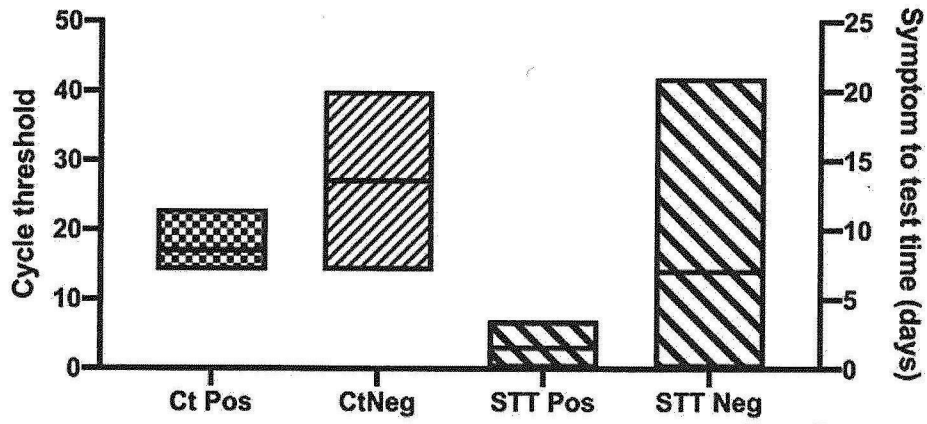
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Figure 1

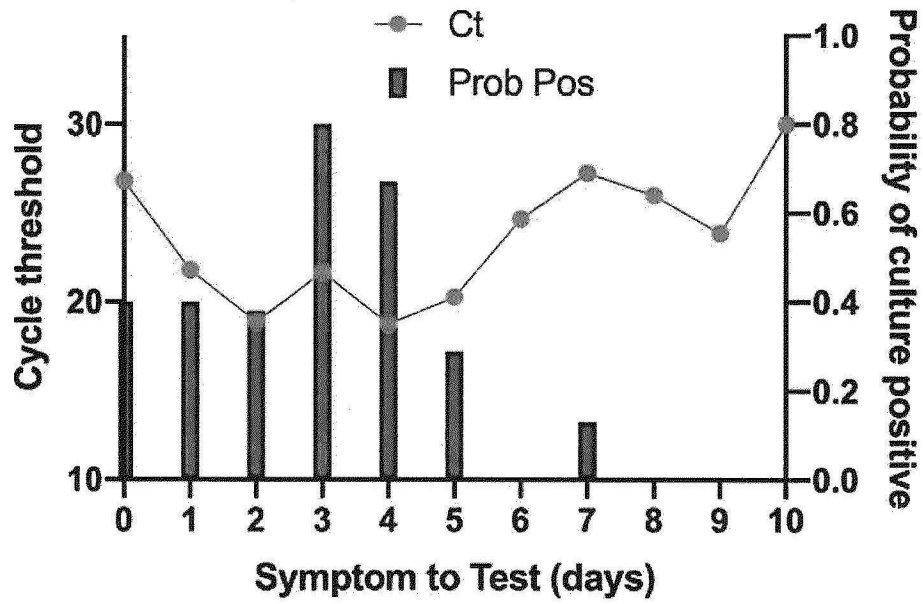


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Figure 2



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Bastien, Nathalie (PHAC/ASPC)

From: Li, Yan (PHAC/ASPC)
Sent: 2020-04-14 10:19 AM
To: [REDACTED]
Cc: Bastien, Nathalie (PHAC/ASPC)
Subject: RE: propagate VIDO viral culture isolate (COVID-19 virus)

Yes, your calculation is correct. The working solution should be stored at -20C.

Yan

From: [REDACTED] <[REDACTED]@oahpp.ca>
Sent: 2020-04-14 10:15 AM
To: Li, Yan (PHAC/ASPC) <yan.li@canada.ca>
Cc: Bastien, Nathalie (PHAC/ASPC) <nathalie.bastien@canada.ca>
Subject: RE: propagate VIDO viral culture isolate (COVID-19 virus)

Hi Yan,

Thanks for the information , it is very helpful.

Can the working dilution, (1mg/ml) be stored at 4 C or should it be frozen at -20 C ?

Also, in order to get 1 ug/ml solution, I am figuring that I have to use only 1 ul of this solution per ml of the working solution? So, if I am making up 20ml then I only need 20ul?

Thank you,

[REDACTED]

From: Li, Yan (PHAC/ASPC) [mailto:yan.li@canada.ca]
Sent: April 14, 2020 10:57 AM
To: [REDACTED] <[REDACTED]@oahpp.ca>
Cc: Bastien, Nathalie (PHAC/ASPC) <nathalie.bastien@canada.ca>
Subject: RE: propagate VIDO viral culture isolate (COVID-19 virus)

Hi [REDACTED]

If you like , you can double up on this amount (50 ul virus plus 2ml medium)to ensure sufficient cell coverage in the flask.

For 50 mg TPCK trypsin, you could first dissolve in 50 ml medium (1 mg/ml), then, you can use it to prepare working solution.

Hope this will be helpful.

Yan

ATIA - 19(1)

From: [REDACTED]@oahpp.ca>
Sent: 2020-04-13 7:54 AM
To: Li, Yan (PHAC/ASPC) <yan.li@canada.ca>
Cc: Bastien, Nathalie (PHAC/ASPC) <nathalie.bastien@canada.ca>
Subject: RE: propagate VIDO viral culture isolate (COVID-19 virus)

Hi Dr. Li,

I have a question for you regarding the virus propagation. When we perform the 1 hour incubation with the following dilution:

50ul virus plus 950ul MEM+2%FBS+penn/strep+ 1ug/ml of TPCK trypsin for 1 hr, my question is :

Would this volume be enough to cover the whole 72 cm flask surface? It is a total of 1.0 ml volume , is this sufficient? Is there any need for us to possibly double up on this amount, to ensure sufficient cell coverage in the flask? Is there a quick formula for preparing the 1ug/ml TPCK solution, I just want to double check with my calculation to ensure we're using the proper concentration , we have received T1426 TPCK, so it would be the same .

Thanks so much,

[REDACTED]

From: Li, Yan (PHAC/ASPC) [mailto:yan.li@canada.ca]
Sent: March 23, 2020 3:05 PM
To: [REDACTED]@oahpp.ca>; Bastien, Nathalie (PHAC/ASPC) <nathalie.bastien@canada.ca>
Subject: RE: propagate VIDO viral culture isolate (COVID-19 virus)

Hi [REDACTED]

Here is how we propagate a viral stock:

We culture Vero E6 cells in MEM+10%FBS+Penn/strep at 37C/5%CO2 in T75 flask. When cells grow to 80-90% confluence, cells were infected with 50ul virus plus 950ul MEM+2%FBS+penn/strep+ 1ug/ml of TPCK trypsin for 1 hr. Then inoculum was removed and replaced with 20 ml of fresh MEM+2%FBS+penn/strep+ 1ug/ml of TPCK trypsin. CPE showed up at day 3 postinfection.

TPCK trypsin is from Sigma. Cat#: T1426-50mg. It is lyophilized. We directly dissolve it in MEM.

Yan

[REDACTED]

From: [REDACTED]@oahpp.ca>
Sent: 2020-03-23 1:10 PM
To: Bastien, Nathalie (PHAC/ASPC) <nathalie.bastien@canada.ca>
Cc: Li, Yan (PHAC/ASPC) <yan.li@canada.ca>
Subject: propagate VIDO viral culture isolate (COVID-19 virus)

Hi Nathalie,

I just have a few questions for you re. the COVID -19 virus propagation.

ATIA - 19(1)

We have repassed Vero 76 cells in EMEM with FBS, Pen Strep and Fungizone added, and they are currently starting to grow. We are not familiar with this media, as we don't use it as our primary media for the PRNT West Nile assay.


1. Your current procedure states that TPCK Trypsin (1ug/ml) , is added to the inoculum . We don't use this TPCK at all, can you provide me with the supplier and Catalogue number for this trypsin? Is it in lyophilized or liquid form? From what I see in the procedure, this same concentration of TPCK is then used for the 3 day incubation, should this incubation also be done at 37 C ?
2. After the CPE is observed at 3 days, do we have to perform any freeze-thaw cycles, or is the virus primarily in the 20 mls of media that we have added after the 1 hour incubation?
3. Is it necessary to use this supernatant, and repeat this procedure in order to amplify the growth of virus?

Thanks very much,



Public Health Ontario | Santé publique Ontario

Public Health Laboratory – Toronto | Laboratoire de santé publique – Toronto
661 University Avenue, 20th Floor
Toronto, ON M5G 1M1

@oahpp.ca

*Please note: Public Health Ontario is the new operating name for Ontario Agency for Health Protection and Promotion.
Notez que Santé publique Ontario est le nouveau nom de l'Agence ontarienne de protection et de promotion de la santé.*

Bastien, Nathalie (PHAC/ASPC)

From: Li, Yan (PHAC/ASPC)
Sent: 2020-03-09 9:13 AM
To: [REDACTED]@oahpp.ca
Cc: Bastien, Nathalie (PHAC/ASPC); Li, Yan (PHAC/ASPC)
Subject: propagate VIDO viral culture isolate (COVID-19 virus)

H [REDACTED]

Here is how we propagate a viral stock:

We culture Vero E6 cells in MEM+10%FBS+Penn/strep at 37C/5%CO2 in T75 flask. When cells grow to 80-90% confluence, cells were infected with 50ul virus plus 950ul MEM+2%FBS+penn/strep+ 1ug/ml of TPCK trypsin for 1 hr. Then inoculum was removed and replaced with 20 ml of fresh MEM+2%FBS+penn/strep+ 1ug/ml of TPCK trypsin. CPE showed up at day 3 postinfection.

Hope this helps.

Yan

Yan Li, Ph.D.

Chief, Influenza and Respiratory Viruses Section
National Microbiology Laboratory
Public Health Agency of Canada
Canadian Science Centre for Human and Animal Health
1015 Arlington St., Suite H4050
Winnipeg, MB
Canada R3E 3R2
Phone: 204-789-6045
Fax: 204-789-2082
EMail: yan.li@canada.ca

Bastien, Nathalie (PHAC/ASPC)

From: Li, Yan (PHAC/ASPC)
Sent: 2020-02-25 1:41 PM
To: Gilmour, Matthew (PHAC/ASPC)
Cc: Bastien, Nathalie (PHAC/ASPC); Li, Yan (PHAC/ASPC)
Subject: Vido virus growth

Hi Matt,

I want to let you know that we have grown Vido virus. We have obtained low CT with Corman E assay. We will work with Morag to get sequence.

Yan

Yan Li, Ph.D.

Chief, Influenza and Respiratory Viruses Section
National Microbiology Laboratory
Public Health Agency of Canada
Canadian Science Centre for Human and Animal Health
1015 Arlington St., Suite H4050
Winnipeg, MB
Canada R3E 3R2
Phone: 204-789-6045
Fax: 204-789-2082
EMail: yan.li@canada.ca



Natural Sciences and Engineering
Research Council of Canada

Conseil de recherches en sciences
naturelles et en génie du Canada

350 Albert Street
Ottawa, Canada
K1A 1H5

350, rue Albert
Ottawa, Canada
K1A 1H5

Access to Information and Privacy
Accès à l'information et protection de renseignements personnels
Tel: 613-995-6214

December 10, 2020

[Redacted]

Dear [Redacted]

PROTECTED

Your File Votre référence

Our File Notre référence
A-2020-00029

This is in response to your access to information request received by our office on December 8, 2020, made pursuant to the *Access to Information Act* (the *Act*) which reads as follows:

"All records 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. To clarify, I am requesting all such records that are in the possession, custody or control of your institution Canada (for example: downloaded to a computer, printed in hard copy, etc.). The known or estimated error rate (both false positives and false negatives), of PCR testing to test for SARS-COV-2. This can include reference to any studies The known or estimated error rate (both false positives and false negatives), of antibody testing to check for immunity to SARS-COV-2. This can include references to any studies Whether vaccine manufacturers have been indemnified (rendered legally immune from lawsuit), for any vaccines they provide related to SARS-COV-2 Whether any vaccine injury compensation plan will be established (or has been established), for people who are injured or killed by vaccines to treat SARS-COV-2"

Please be advised that the Natural Sciences and Engineering Research Council of Canada (NSERC) does not have any records that respond to your request.

Please note that you are entitled to file a complaint with the Information Commissioner of Canada within sixty days of receipt of this response. Notice of complaint should be addressed to:

Information Commissioner of Canada
30 Victoria Street, Gatineau, QC K1A 1H3
Telephone: (613) 995-2410 (National Capital Region) 1-800-267-0441 (Toll-free)

Should you require additional information concerning your request, do not hesitate to contact me at 343-571-9689 or by email at Julie.Bourbonnais@nserc-crsng.gc.ca.

Sincerely,

Julie Bourbonnais



Digitally signed by Julie Bourbonnais
DN: cn=Julie Bourbonnais, o=NSEERC,
ou=Scholarships & Fellowships,
email=julie.bourbonnais@nserc-
crsng.gc.ca, c=CA
Date: 2020.12.10 11:32:08 -0500'

Julie Bourbonnais
Manager, ATIP & Governance | Gestionnaire, AIPRP et gouvernance
Secretariat | Secrétariat
Natural Sciences and Engineering Research Council of Canada | Conseil de recherches en sciences
naturelles et en génie du Canada



Canadian Institutes of Health Research

160 Elgin Street, 9th Floor
Address Locator 4809A
Ottawa, Ontario K1A 0W9

Instituts de recherche en santé du Canada

160, rue Elgin, 9^e étage
Indice de l'adresse 4809A
Ottawa (Ontario) K1A 0W9

Institute of Aboriginal Peoples' Health

Institute of Aging

Institute of Cancer Research

Institute of Circulatory and Respiratory Health

Institute of Gender and Health

Institute of Genetics

Institute of Health Services and Policy Research

Institute of Human Development, Child and Youth Health

Institute of Infection and Immunity

Institute of Musculoskeletal Health and Arthritis

Institute of Neurosciences, Mental Health and Addiction

Institute of Nutrition, Metabolism and Diabetes

Institute of Population and Public Health

Institut de la santé des Autochtones

Institut du vieillissement

Institut du cancer

Institut de la santé circulatoire et respiratoire

Institut de la santé des femmes et des hommes

Institut de génétique

Institut des services et des politiques de la santé

Institut du développement et de la santé des enfants et des adolescents

Institut des maladies infectieuses et immunitaires

Institut de l'appareil locomoteur et de l'arthrite

Institut des neurosciences, de la santé mentale et des toxicomanies

Institut de la nutrition, du métabolisme et du diabète

Institut de la santé publique et des populations

December 15, 2020

Ref: A-2020-0029



By Email



On December 8, 2020, the Canadian Institutes of Health Research received your request for information made under the *Access to Information Act* for the following:

“All records 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. To clarify, I am requesting all such records that are in the possession, custody or control of your institution Canada (for example: downloaded to a computer, printed in hard copy, etc.). The known or estimated error rate (both false positives and false negatives), of PCR testing to test for SARS-COV-2. This can include reference to any studies The known or estimated error rate (both false positives and false negatives), of antibody testing to check for immunity to SARS-COV-2. This can include references to any studies Whether vaccine manufacturers have been indemnified (rendered legally immune from lawsuit), for any vaccines they provide related to SARS-COV-2 Whether any vaccine injury compensation plan will be established (or has been established), for people who are injured or killed by vaccines to treat SARS-COV-2”

I regret to inform you that The Canadian Institutes of Health Research does not have any records under our control relating to your request. COVID-19 academic publications resulting from CIHR-funded research can be found on our website at <https://cihr-irsc.gc.ca/e/51948.html> and Information on the publication of research findings can be found in the Tri-Agency Open Access Policy on Publications [here](#).

Please be advised that you are entitled to complain to the Information Commissioner concerning the processing of your request within 60 days after the day that you become aware that grounds for a complaint exist. In the event you decide to avail yourself of this right, your notice of complaint should be addressed to:

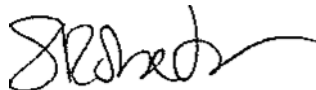


The Information Commissioner of Canada
30 Victoria Street, 7th Floor
Gatineau, Quebec K1A 1H3

You may obtain additional information on the complaint process by visiting the website of the Office of the Information Commissioner at www.oic-ci.gc.ca/en/submitting-complaint.

This completes our processing of your request. If you have any questions concerning your request, please contact me, by email at ATIPCoordinator@cihr-irsc.gc.ca.

Sincerely,

A handwritten signature in black ink, appearing to read 'S. Robertson', with a long horizontal flourish extending to the right.

Sharon Robertson
ATIP Coordinator

CSC 23485 First UK COVID-19 vaccine approved Pfizer/BioNTech



From: frances leader <franleader@hotmail.co.uk>

Sent: 03 December 2020 16:29

To: MHRA Customer Services <MHRACustomerServices@mhra.gov.uk>

Subject: CSC 23485 First UK COVID-19 vaccine approved Pfizer/BioNTech

Dear Sirs,

As you have approved the Pfizer/BioNTech vaccine for distribution to UK citizens from next week I would appreciate a copy of the Vaccine insert detailing full ingredients, all known side effects & all other medicine safety information which would normally be available with any medication.

A FOIA to Public Health Scotland recommended that you should be able to supply this information as they cannot.



MHRA Customer Services <MHRACustomerService
s@mhra.gov.uk>

Tue 08/12/2020 13:46

To: You



Our reference: CSC 23485

Dear Fran Leader,

Thank you for your email dated 3/12.

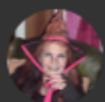
We recently published information on the product and advise you review this at the following link of our website page below that contains the patient information leaflet and summary of product characteristics.

<https://www.gov.uk/government/news/uk-medicines-regulator-gives-approval-for-first-uk-covid-19-vaccine>

Here is a direct link to the PDF which would answer your query:

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/940566/Information_for_UK_recipients_on_Pfizer_BioNTech_COVID-19_vaccine.pdf

CSC 23485 First UK COVID-19 vaccine approved Pfizer/BioNTech



frances leader

Wed 09/12/2020 20:13

To: MHRA Customer Services



Dear Adam,

You took so long to answer my email I have already seen the docs you offer below. Plus the advice distributed to healthcare professional.

Can you give me any idea of what is in the active ingredient BNT162b2 RNA?

It is not specified anywhere that I have seen.

Thanks for your attention to this matter.

Kind regards,

Fran Leader

CSC 23485 First UK COVID-19 vaccine approved Pfizer/BioNTech



MHRA Customer Services <MHRACustomerService
s@mhra.gov.uk>

Fri 11/12/2020 12:00

To: You



Our reference: CSC 23485

Dear Fran Leader,

Thank you for your email. Apologies for the delay in response.
BNT162b2 RNA is embedded in lipid nanoparticles.

COVID-19 mRNA Vaccine BNT162b2 is highly purified single-stranded, 5'-capped messenger RNA (mRNA) produced by cell-free in vitro transcription from the corresponding DNA templates, encoding the viral spike (S) protein of SARS-CoV-2.

Should you require any further advice or assistance on this matter please feel free to call us on 0203 080 6000 or reply to this email.

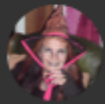
Our opening hours are Mon – Fri 9am to 5pm (excluding UK Public Holidays)

CSC 23485 First UK COVID-19 vaccine approved Pfizer/BioNTech



Our Reference: CSC 23485 Dear Frances Leader, Thank you for your email. We have revi...

Mon 14/12/2020 14:13



frances leader

Fri 11/12/2020 20:47

To: MHRA Customer Services



Thank you Adam!

I would like you to confirm that the DNA template has come from a computer generated genomic sequence first notified to WHO by China rather than an isolated virus from an infected person.

thank you!

Frances Leader

CSC 23485 First UK COVID-19 vaccine approved Pfizer/BioNTech



MS

MHRA Customer Services <MHRACustomerService
s@mhra.gov.uk>

Mon 14/12/2020 14:13

To: You



Our Reference: CSC 23485

Dear Frances Leader,

Thank you for your email.

We have reviewed your request and this has been referred onward for consideration.

In the meantime, should you have any other questions or requests please feel free to call us on 0203 080 6000 or email at info@mhra.gov.uk

Our opening hours are Mon – Fri 9am to 5pm (excluding UK Public Holidays)

Kind regards

CSC 23485 First UK COVID-19 vaccine approved Pfizer/BioNTech

To: You

Our reference: CSC 23485

Dear Frances Leader,

Thank you for your email.

The information is in the Public Assessment Report: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/944544/COVID-19_mRNA_Vaccine_BNT162b2_UKPAR_PFIZER_BIONTECH_15Dec2020.pdf

A quality target product profile for the finished product has been established taking into consideration the World Health Organization's "WHO Target Product Profiles for COVID-19 Vaccines".

The DNA template used does not come directly from an isolated virus from an infected person.

Should you require any further advice or assistance on this matter please feel free to call us on 0203 080 6000 or reply to this email.

CSC 23485 First UK COVID-19 vaccine approved Pfizer/BioNTech



MHRA Customer Services <MHRACustomerService
s@mhra.gov.uk>

Mon 21/12/2020 10:46

To: You



Our reference: CSC 23485

Dear Frances Leader,

Just to add some further information:

The DNA template(severe acute respiratory syndrome coronavirus 2, GenBank: MN908947.3)
was generated via a combination of gene synthesis and recombinant DNA technology.

Should you require any further advice or assistance on this matter please feel free to call us on
0203 080 6000 or reply to this email.

Our opening hours are Mon – Fri 9am to 5pm (excluding UK Public Holidays)

With regards



Centers for Disease Control
and Prevention (CDC)
Atlanta GA 30333
December 30, 2020

SENT VIA EMAIL

[REDACTED]
Dear Mr. [REDACTED]

This letter is our final response 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 December 20, 2020, assigned #21-00394-FOIA, for:

All records in the possession, custody or control of The Centers for Disease Control (CDC) describing the isolation of a SARS-COV-1 viruses well as any of the other common cold associated coronaviruses, 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-1 or any of the other common cold associated coronaviruses 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 CDC or that pertain to work done by The CDC. My request includes any sort of record, for example (but not limited to any published peer-reviewed study that the CDC 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 record with certainty (i.e. title, author(s), date, journal, where the public may access it).

A search of our records failed to reveal any documents pertaining to your request.

You may contact our FOIA Public Liaison at 770-488-6277 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.

If you are not satisfied with the response to this request, you may administratively appeal by writing to the Deputy Agency Chief FOIA Officer, Office of the Assistant Secretary for Public Affairs, U.S. Department of Health and Human Services, Hubert H. Humphrey Building, 200 Independence Avenue, Suite 729H, Washington, D.C. 20201. You may also transmit your appeal via email to FOIARequest@psc.hhs.gov. Please mark both your appeal letter and envelope "FOIA Appeal." Your appeal must be postmarked or electronically transmitted by Tuesday, March 30, 2021.

You may wish to visit the following link for publications regarding the isolation of SARS-CoV-1 and other human coronaviruses: <https://pubmed.ncbi.nlm.nih.gov/>.

Sincerely,



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