



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](mailto: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)**

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**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]



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,  
[REDACTED]

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

Jared Bullard MD<sup>1,2,3</sup>, Kerry Dust PhD<sup>1</sup>, Duane Funk MD<sup>4,5</sup>, James E. Strong MD, PhD<sup>2,3,4</sup>, David Alexander PhD<sup>1,3</sup>, Lauren Garnett BSc<sup>3,4</sup>, Carl Boodman MD<sup>3</sup>, Alexander Bello PhD<sup>3,4</sup>, Adam Hedley BSc<sup>1</sup>, Zachary Schiffman BSc<sup>3,4</sup>, Kaylie Doan BSc<sup>4</sup>, , Nathalie Bastien PhD<sup>3,4</sup>, Yan Li PhD<sup>3,4</sup>, Paul G. Van Caesele MD<sup>1,2,3</sup> and Guillaume Poliquin MD, PhD<sup>2,3,4</sup>

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**Summary:** Respiratory samples from COVID-19 patients with  $\geq 8$  days of symptoms and a SARS-CoV-2 E gene RT-PCR Ct value  $\geq 24$  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 TCID<sub>50</sub>/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% CO<sub>2</sub> 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 TCID<sub>50</sub>/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,  $p<0.001$ ), 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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## References:

1. Wölfel R, Corman VM, Guggemos W, *et al.* Virological assessment of hospitalized patients with COVID-19. Nature [published online April 1, 2020]. Available from: <https://doi.org/10.1038/s41586-020-2196-x>
2. He X, Lau EHY, Wu P, *et al.* Temporal dynamics in viral shedding and transmissibility of COVID-19. Nature Medicine [published online April 15, 2020]. Available from: <https://doi.org/10.1038/s41591-020-0869-5>
3. Ip DKM, Lau LLH, Chan KH, *et al.* The dynamic relationship between clinical symptomatology and viral shedding in naturally acquired seasonal and pandemic influenza virus infection. Clin. Infect. Dis. February 2016; 62(4): 431-37.
4. Falsey AR, Formica MA, Treanor JJ *et al.* Comparison of quantitative reverse transcription-PCR to viral culture for assessment of respiratory syncytial virus shedding. J. Clin. Micro. September 2003; 41(9): 4160-65.
5. Corman VM, Landt O, Kaiser M, *et al.* Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro. Surveill. 2020 Jan; 25(3). doi: 10.2807/1560-7917.ES.2020.25.3.2000045.
6. Ramakrishnan MA. Determination of 50% endpoint titer using a simple formula. World J

7. Government of Canada, Public Health Agency of Canada (PHAC). Coronavirus disease (COVID-19):

For health professionals. Accessed April 24, 2020. <https://www.canada.ca/en/public-health/services/diseases/2019-novel-coronavirus-infection/health-professionals.html>.

8. Centers for Disease Control and Prevention. Symptom-Based Strategy to Discontinue Isolation for Persons with COVID-19. Accessed May 5, 2020. <https://www.cdc.gov/coronavirus/2019-ncov/community/strategy-discontinue-isolation.html>.

9. Shared Health Manitoba. Provincial COVID-19 resources for health-care providers and staff. Accessed on April 24, 2020. <https://sharedhealthmb.ca/covid19/providers/>.

10. Institut national de santé publique Québec (INSPQ). COVID-19 (coronavirus) public health expertise and reference centre. Accessed April 24, 2020. <https://www.inspq.qc.ca/en>.

11. Public Health Ontario. COVID-19 Health Care Resources. Public Health Ontario. Accessed April 24, 2020. <https://www.publichealthontario.ca/en/diseases-and-conditions/infectious-diseases/respiratory-diseases/novel-coronavirus/health-care-resources>.

12. BC Centre for Disease Control (BCCDC). COVID-19 Care. Accessed April 24, 2020. <http://www.bccdc.ca/health-professionals/clinical-resources/covid-19-care>.



13. Strong JE, Feldmann H. The Crux of Ebola Diagnostics. J Infect Dis. 2017;216(11):1340 - 1342.

doi:10.1093/infdis/jix490

14. Centers for Disease Control and Prevention. Research Use Only 2019-Novel Coronavirus (2019-nCoV) Real-time RT-PCR Primer and Probe Information (CDC N1 gene). Accessed May 15, 2020.

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

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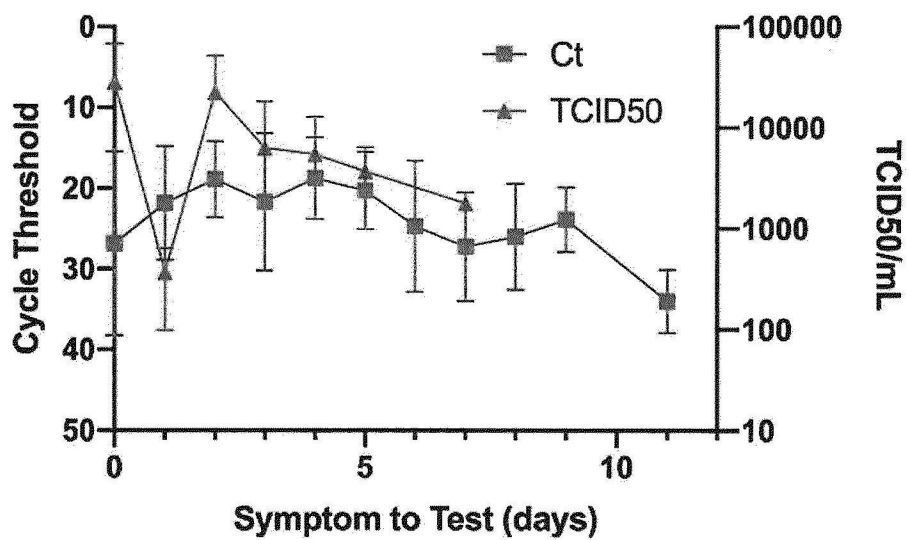
### Figure Legends:

Figure 1: SARS-CoV-2 viral dynamics as expressed by E gene RT-PCR Cycle threshold (Ct) value and cell culture TCID<sub>50</sub>/mL, over time (days). Squares represent Ct values while triangles reflect TCID<sub>50</sub>.

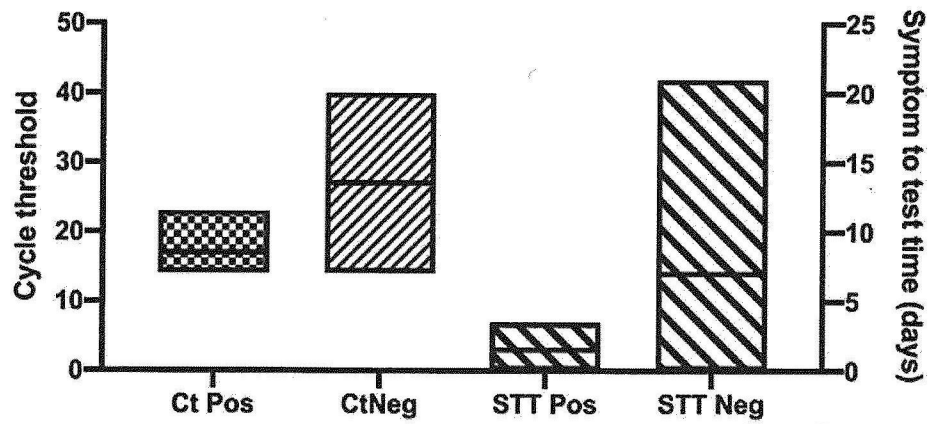
Figure 2: SARS-CoV-2 E gene RT-PCR Cycle Threshold (Ct) values and symptom to test time (STT) in samples that were culture positive (Ct +, STT +), or negative (Ct -, STT -). Positive SARS-CoV-2 culture samples had a significantly lower Ct when compared to culture negative samples (17 [16-18] vs 27 [22-33],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

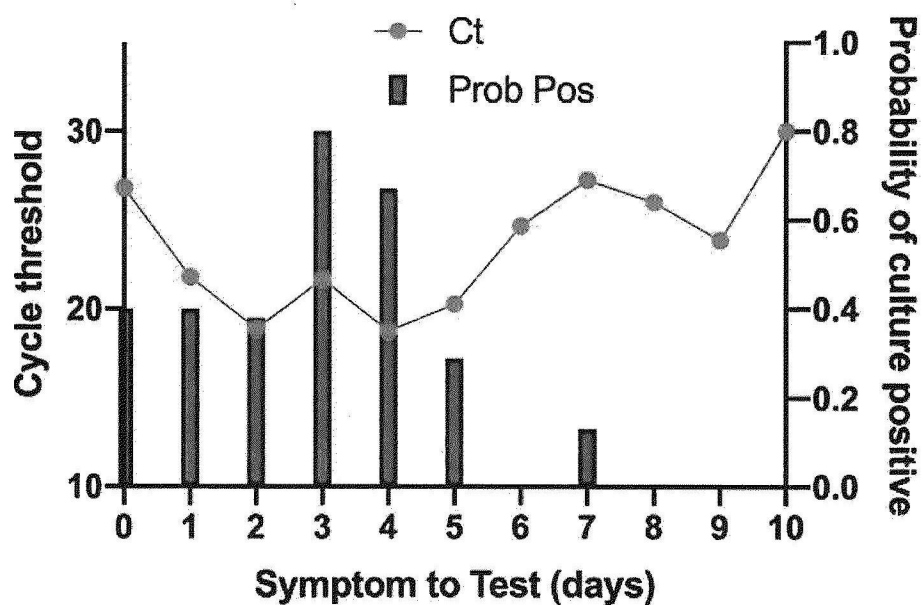
Figure 3: Comparison of symptom onset to test (days) to the probability of successful cultivation on Vero cells and SARS-CoV-2 E gene RT-PCR Cycle threshold (Ct) value. Ct values are represented by the line graph with circles. Probability of SARS-CoV-2 culture is shown by the bar graph.

Figure 1



**Figure 2**







**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]@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]@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

**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)

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.

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

Yan

**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.

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  
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Toronto, ON M5G 1M1

[publichealth@oahpp.ca](mailto:publichealth@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)**

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**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/%%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.**

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**Bastien, Nathalie (PHAC/ASPC)**

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**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  
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