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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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.

Figure 1

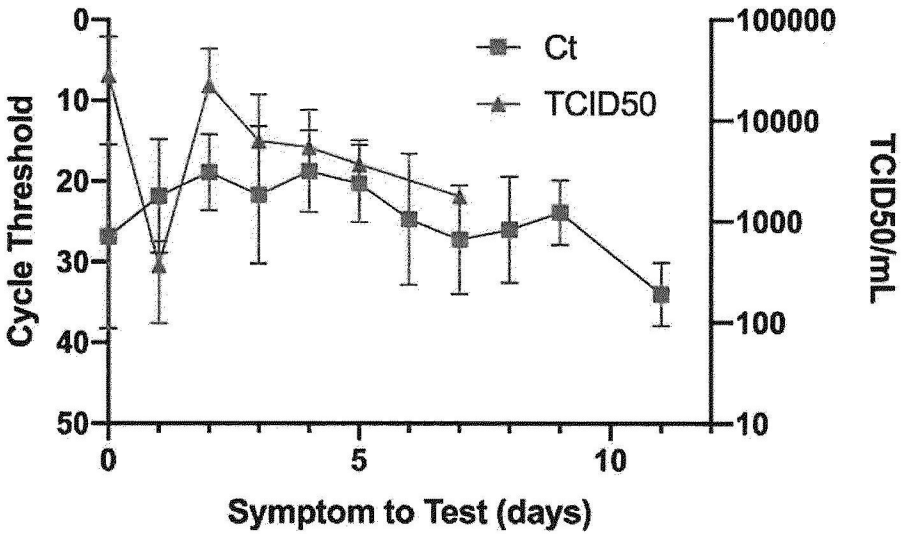
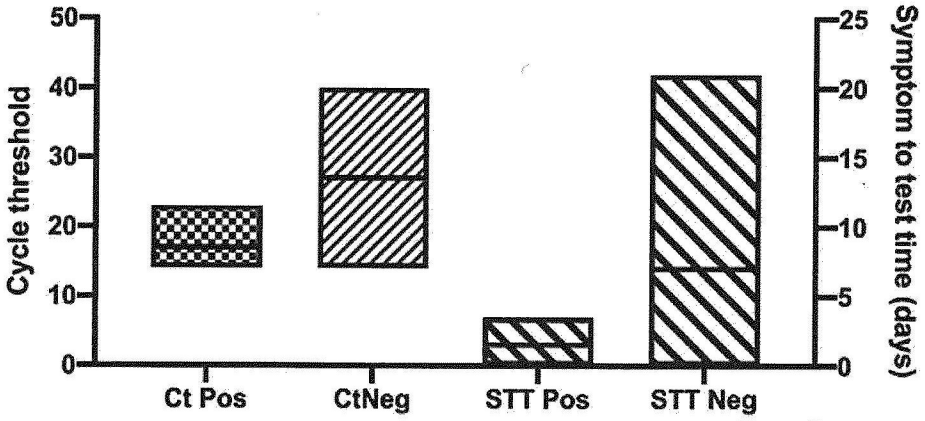
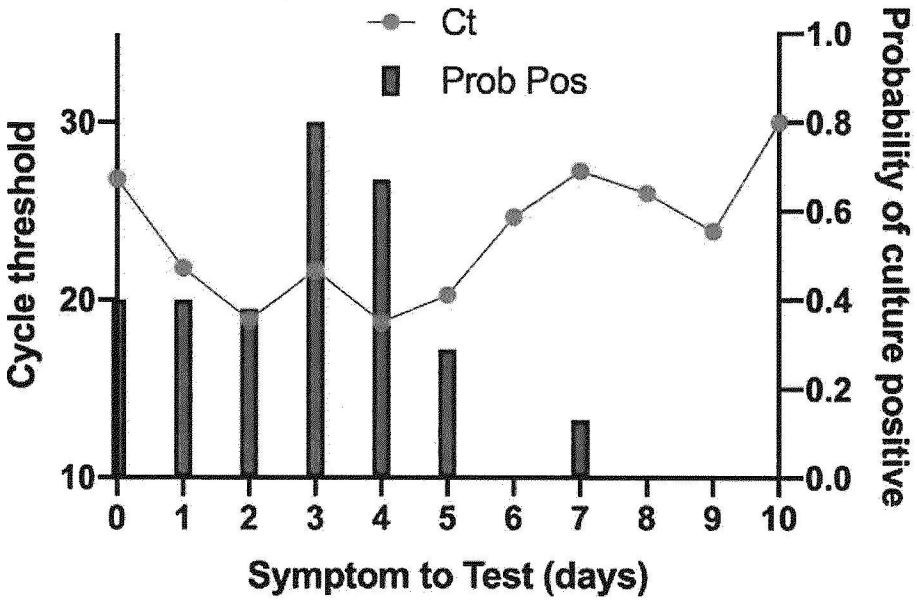


Figure 2





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 I 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 I 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,

[redacted]

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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/%%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 I 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

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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

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