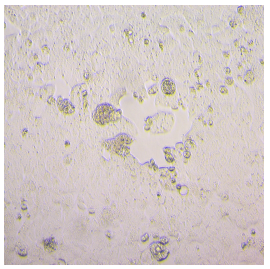
Feb 12, 2020

Culture of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; f.2019-nCoV)



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Public Health Virology, Fo… Coronavirus Method Deve…

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We use this protocol and it's working

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Abstract

We briefly describe a method to inoculate a susceptible cell line with a human patient sample in order to culture the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the cause of coronavirus disease, COVID-19.

Clinical samples including nasopharyngeal swabs and aspirates were inoculated onto confluent monolayers of African green monkey kidney Vero C1008, clone E6 cells (ATCC®-CRL-1586) grown in Opti-Mem reduced serum growth medium supplemented with 3% foetal bovine serum in polystyrene, flat-sided, screw-cap 3 mL cell culture tubes.

This work was conducted in Queensland under PC3 laboratory conditions by experienced scientists.

Materials

MATERIALS

- Nunc™ Cell Culture Tubes, 3mL **Thermo Fisher Catalog #**156758
- Opti-MEM™ I Reduced Serum Medium **Thermo Fisher Catalog #**31985088
- Acrodisc® syringe filters13 mm Dia 0.2 µm Pore Size **Cole-Parmer Catalog #**4602
- Foetal Bovine Serum (FBS) Triple 0.1 µm Sterile Filtered 500 ml Australian Origin **Serana Catalog #**FBS-AU-015

Safety warnings

This work was conducted in Queensland under PC3 laboratory conditions by experienced virologists.

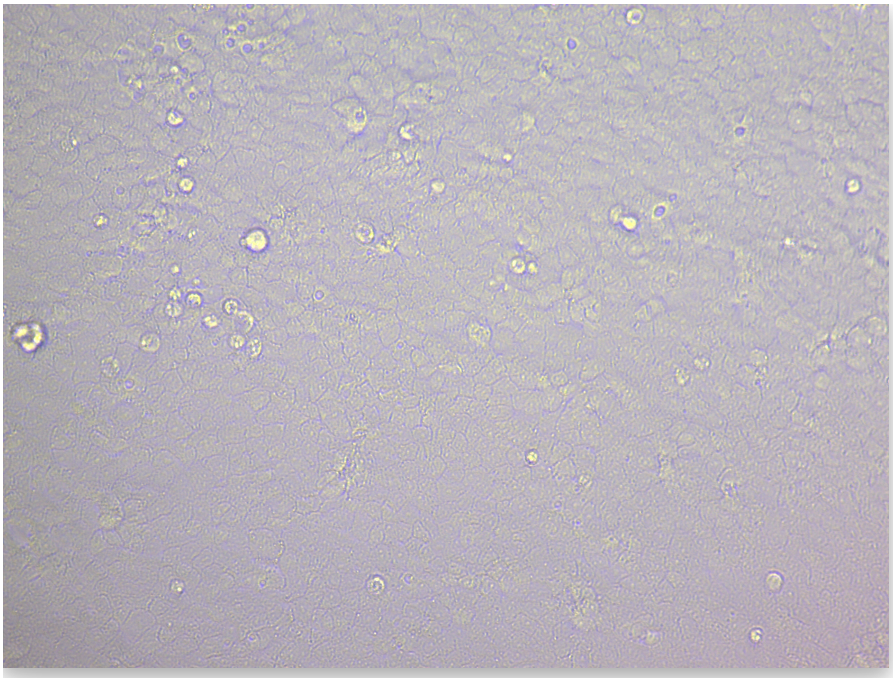
Before start

- this protocol assumes extensive experience in cell culture, isolation of human viral pathogens, handling of biological samples of human origin, working under PC3 laboratory conditions, disposal of biological, chemical and infectious wastes, knowledge and trained use of appropriate PPE and ongoing, documented and up to date training for work under all of these conditions.
- this protocol assumes culture vessels of cells have been expertly maintained, recently and appropriately split and that cells for inoculation are in an active growth phase.

Specimen and uninfected cell preparation

1 Cell culture tubes were moved from the 37ºC incubator to a Class II Biosafety cabinet, within a PC3 laboratory environment.

Growth medium from previously prepared Vero E6 tubes was discarded to waste.



A confluent, uninfected monolayer culture of Vero E6 cells in Opti-MEM (no FBS).
Source: Dr. Alyssa Pyke, Public Health Virology Laboratory,
Forensic and Scientific Services, Queensland.
08FEB2020

2 Clinical samples were prepared by diluting in Opti-Mem® reduced serum growth medium without foetal bovine serum (FBS) and filtering through a 0.2 µM, 13 mm Acrodisc® filter (Bio-Strategy Laboratory Products, Australia).

Inoculation of Vero E6 cell monolayers

3 150-200 µL of filtered patient material was inoculated onto separate confluent cell monolayers along with a negative control tube (150-200 µL of Opti-Mem alone).

NOTE:
Fresh specimens are best for succesful viral culture.

Samples were absorbed onto cells by incubating tubes for 1 hr at 37ºC before cultures were re-fed with 2 mL of pre-warmed (37ºC) Opti-Mem® reduced serum growth medium.

NOTE:
It can be informative to collect a 200 µL sample at this point as a Day 0 value to test alongside other culture samples from Day 2 onwards.

Culture incubation and observation

4 Cultures were incubated for 2-7 days until signs of cytopathic effect (CPE) was observed.

In our hands, cultures that were inoculated with patient sample extracts which, at original testing produced C_Ts of approximately 20 cycles, developed signs of CPE within 3 days post-inoculation.

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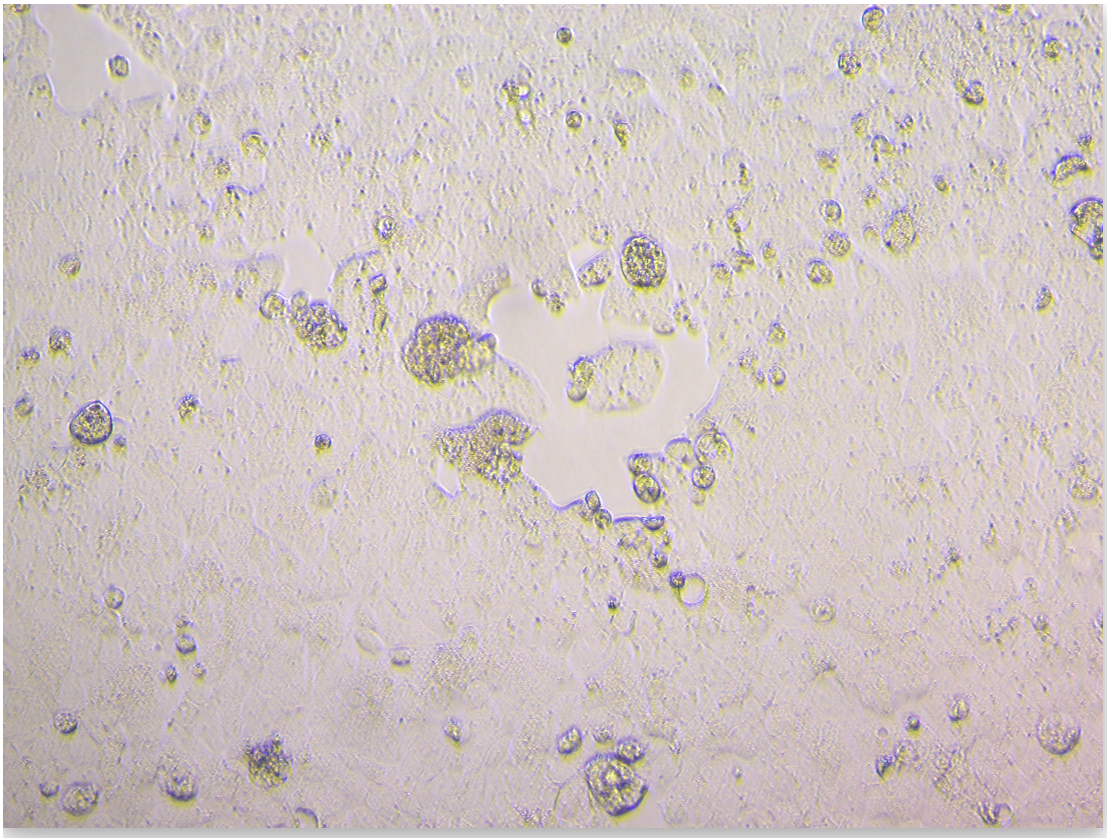

An example of a **SARS-CoV-2-infected** monolayer culture of Vero E6 cells demonstrating focal CPE.
Source: Dr. Alyssa Pyke, Public Health Virology Laboratory, Forensic and Scientific Services, Queensland.
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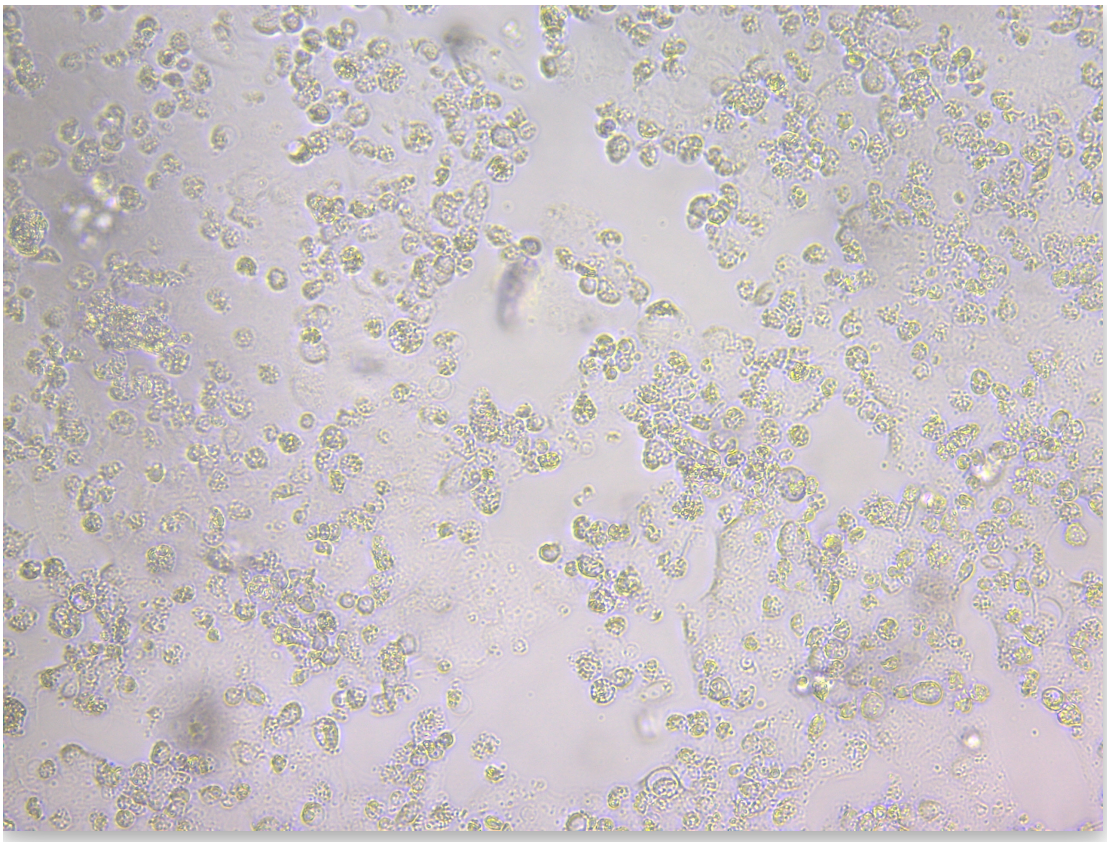
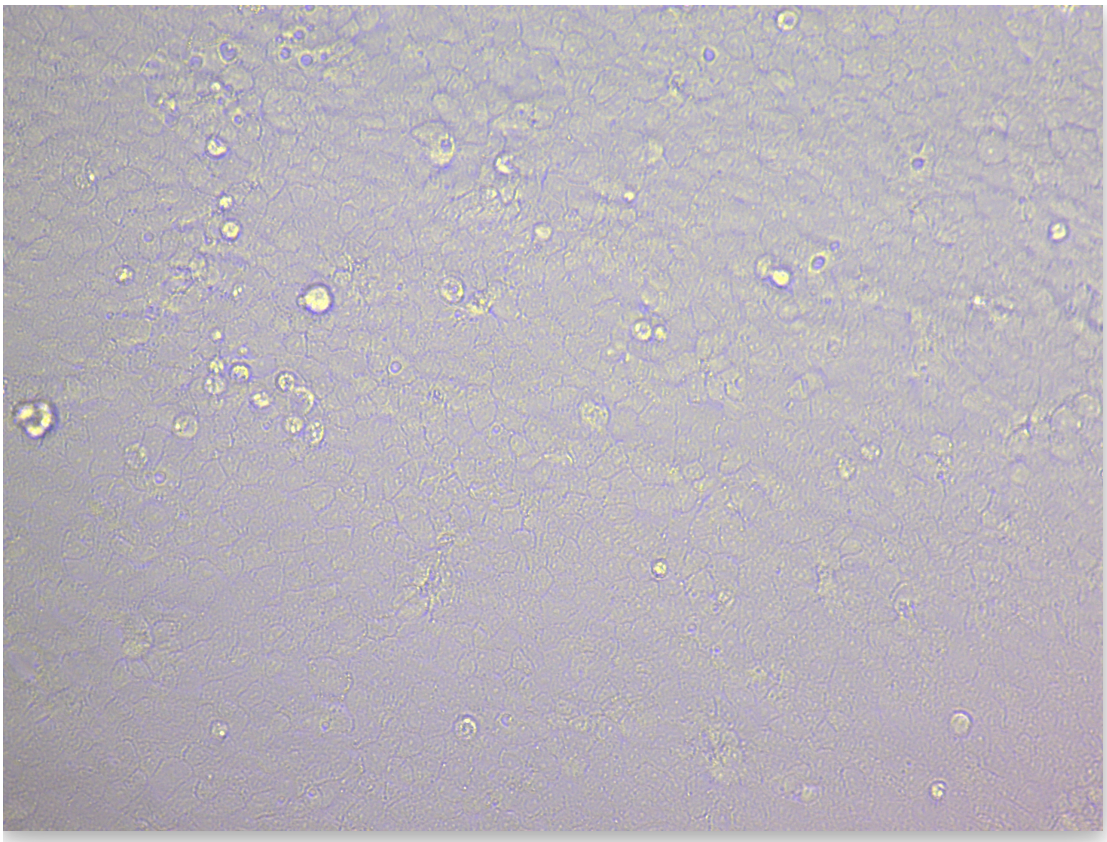


An example of much more advanced CPE in a **SARS-CoV-2-infected** monolayer culture of Vero E6 cells demonstrating widespread CPE.
Source: Dr. Alyssa Pyke, Public Health Virology Laboratory, Forensic and Scientific Services, Queensland.
08FEB2020



A confluent, **uninfected monolayer** culture of Vero E6 cells in Opti-MEM.
Source: Dr. Alyssa Pyke, Public Health Virology Laboratory,
Forensic and Scientific Services, Queensland.
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Confirmation of a succesful virus culture

6 A cell culture is suspected of hosting virus replication based on the presence of CPE including damage to the monolayer, cell-clearing and morphological changes.

Suspected cultures were further confirmed using the Corman *et al*. **E** and Northill *et al*. **ORF1ab** novel coronavirus reverse-transcription real-time polymerase chain reaction (RT-rPCRs) tests.

Samples taken from a suspected culture are expected to contain levels of virus that exceed those present in the original clinical sample so we would expect to see C_T values lower than those obtained from testing the original clinical sample extract by RT-rPCRs.

SLOW CULTURES: If the C_T value from an extract of the first passage of a SARS-CoV-2 culture is the same or higher (suggesting a lower viral load) than the C_T value from the RT-rPCR of the original clinical sample, further investigation will be required to ensure the culture has

biologically amplified virus. Consideration should be given to whether such a result is due to the detection of viral remnants from the inoculum rather than virus amplification.

Actions to consider in the absence of obvious CPE

7 In the event low-levels of the virus were not detected by observation of CPE in the first isolation attempt, negative cell culture supernatants should be further passaged onto fresh Vero E6 monolayers up to 3 times with testing as described in Step 6.