At all times local, regional, and national laws and guidelines will be adhered to.

📏 Edit this document to make it better 📏

Corona virus detection

Open source experiment to recreate the comparison of PCR primers for SARS-CoV-2 detection from this paper.

This will only be conducted using samples from the experimenter.

If the experimenter thinks they're infected this experiment must be carried out at home / their place of self isolation.

If the experimenter has no reason to believe they're sick then they could do this in a community biolab though perhaps they'd not do this experiment and want to save the reagents (as they're not that cheap).

At all times will local, regional, and national laws and guidelines be adhered to. This document is open for anyone to improve it.

Parent folder:

https://drive.google.com/open?id=1eSJD2E7eWmZNh8z -0KnHD6FcQpBiBWj

Related projects:

https://groups.google.com/forum/#!msg/diybio/F_y2WsfEJog

Corona Virus Tech Handbook

https://app.jogl.io/project/118

https://groups.google.com/forum/#!topic/london-hack-space/Bw4Hn6Ej-ql

Slack groups:

http://biohackspace.org/slack join the #corona channel

https://jogl-covid-19.slack.com join the #repro-jung2020feb-primer-comparison channel

Tasks remaining

- Request advice from respiratory doctor regarding how to take samples
- clearly describe all the steps in the protocol.
 - Including reagents, equipment, volumes, decision flow etc needed
- find sources of reagents including links to suppliers and prices
 - Struggling to find a supplier of RT-PCR (WELLS BIO Inc.). Their website only lists kits that include RT-PCR as tests for things like Zika, HPV etc.
 - Have contacted WELLS BIO Inc.
 - Have contacted authors requesting link to kit
- anything else?

Recreating comparison of primer sets for SARS-CoV-2 detection

Paper to recreate

https://www.biorxiv.org/content/10.1101/2020.02.25.964775v1.full

Sample prep: Uses QIAamp viral RNA extraction Kit

Detection: 300 nM of primers and probes for the target detection. The qRT-PCR was performed with a CFX 96 touch real-time PCR detection system (Bio-rad, Hercules, CA, USA). The qRT-PCR conditions applied in this study were programmed as follows: UNG incubation, RT incubation, and enzyme activation were serially performed at 25 °C for 2 minutes, at 55 °C for 10 minutes, at 94 °C for 3 minutes respectively. Thermal cycling was then performed at 94 °C for 15 seconds (denaturation), and at 60 °C for 30 seconds (annealing and amplification) for forty-five cycles.

Sequences used

| Target | Country | Name | Ty pe | Sequence (5' → 3') | Position | Ref | Source |
|--------|--------------|---------------------|----------|---------------------------------|---------------|------|--------------------|
| N | China | N-F | F | GGG GAA CTT CTC CTG CTA GAA T | 28881 - 28902 | [17] | COVID-19-3 \$4.18 |
| | | N-R | R | CAG ACA TTT TGC TCT CAA GCT G | 28958 - 28979 | | COVID-19-4 \$4.18 |
| | | N-P | Р | TTG CTG CTG CTT GAC AGA TT | 28934 - 28953 | | |
| | Hong Kong | HKU-NF | F | TAA TCA GAC AAG GAA CTG ATT A | 29145 - 29166 | [9] | TODO: get supplier |
| | | HKU-NR | R | CGA AGG TGT GAC TTC CAT G | 29235 - 29254 | | TODO: get supplier |
| | | HKU-NP | Р | GCA AAT TGT GCA ATT TGC GG | 29177 - 29196 | | |
| | Japan | NIID_2019-nCOV_N_F2 | F | AAA TTT TGG GGA CCA GGA AC | 29125 - 29144 | [18] | TODO: get supplier |
| | | NIID_2019-nCOV_N_R2 | R | TGG CAG CTG TGT AGG TCA AC | 29263 - 29282 | | TODO: get supplier |
| | | NIID_2019-nCOV_N_P2 | Р | ATG TCG CGC ATT GGC ATG GA | 29222 - 29241 | | |
| | Thailand | WH-NIC N-F | F | CGT TTG GTG GAC CCT CAG AT | 28320 - 28339 | [19] | TODO: get supplier |
| | | WH-NIC N-R | R | CCC CAC TGC GTT CTC CAT T | 28358 - 28376 | | TODO: get supplier |
| | | WH-NIC N-P | Р | CAA CTG GCA GTA ACC A | 28341 - 28356 | | |
| | USA | 2019-nCoV_N1-F | F | GAC CCC AAA ATC AGC GAA AT | 28287 - 28306 | [20] | TODO: get supplier |
| | | 2019-nCoV_N1-R | R | TCT GGT TAC TGC CAG TTG AAT CTG | 28335 - 28358 | | TODO: get supplier |
| | | 2019-nCoV_N1-P | Р | ACC CCG CAT TAC GTT TGG TGG ACC | 28309 - 28332 | | |
| | | 2019-nCoV_N2-F | F | TTA CAA ACA TTG GCC GCA AA | 29164 - 29183 | | TODO: get supplier |

| | | 2019-nCoV_N2-R | R | GCG CGA CAT TCC GAA GAA | 29213 - 29230 | | TODO: get supplier |
|----------|--------------|------------------|---|--|---------------|------|--------------------|
| | | 2019-nCoV_N2-P | Р | ACA ATT TGC CCC CAG CGC TTC AG | 29188 – 29210 | | |
| | | 2019-nCoV_N3-F | F | GGG AGC CTT GAA TAC ACC AAA A | 28681 - 28702 | | TODO: get supplier |
| | | 2019-nCoV_N3-R | R | TGT AGC ACG ATT GCA GCA TTG | 28732 - 28752 | | TODO: get supplier |
| | | 2019-nCoV_N3-P | Р | AYC ACA TTG GCA CCC GCA ATC CTG | 28704 - 28727 | | |
| RdRp/Orf | China | ORF1ab-F | F | CCC TGT GGG TTT TAC ACT TAA | 13342 - 13362 | [17] | TODO: get supplier |
| | | ORF1ab-R | R | ACG ATT GTG CAT CAG CTG A | 13442 - 13460 | | TODO: get supplier |
| | | ORF1ab-P | Р | CCG TCT GCG GTA TGT GGA AAG GTT ATG G | 13377 - 13404 | | |
| | Germany | RdRp_SARSr-F | F | GTG ARA TGG TCA TGT GTG GCG G | 15431 - 15452 | [10] | COVID-19-9 \$4.18 |
| | | RdRp_SARSr-R | R | CAR ATG TTA AAS ACA CTA TTA GCA TA | 15505 - 15530 | | COVID-19-10 \$4.94 |
| | | RdRp_SARSr-P2 | Р | CAG GTG GAA CCT CAT CAG GAG ATG C | 15470 - 15494 | | |
| | Hong Kong | HKU-ORF1b-nsp14F | F | TGG GGY TTT ACR GGT AAC CT | 18778 - 18797 | [9] | TODO: get supplier |
| | | HKU-ORF1b-nsp14R | R | AAC RCG CTT AAC AAA GCA CTC | 18889 - 18909 | | TODO: get supplier |
| | | HKU-ORF1b-nsp14P | Р | TAG TTG TGA TGC WAT CAT GAC TAG | 18849 - 18872 | | |

Protocol

Obtain a sample

How to take a sample:

https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories/novel-coronavirus-2019-ncov-sampling-requirements

from "COVID-19: guidance for sampling and for diagnostic laboratories":

https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories

Upper respiratory tract sample options:

- combined nose and throat swab in one collection tube containing universal transport medium OR
- single swab used for throat then nose OR
- individual nose and throat swabs in separate collection tubes OR
- nasopharyngeal aspirate in a universal transport pot

Lower respiratory tract sample in universal container (sputum) if obtainable If the patient is admitted, take a sample for acute serology:

- 5mL in either serum tube or plain (no additive) tube.
- -- Advice from respiratory doc was that you need to wipe the surface by going round and round in the nose for 5 seconds and same for the throat.

Advice from CDC on sample collection said it needed to be done using polyester and not cotton or anything wooden

https://www.who.int/docs/default-source/coronaviruse/uscdcrt-pcr-panel-for-detection-instruct ions.pdf?sfvrsn=3aa07934 2

Swab specimens should be collected only on swabs with a synthetic tip (such as polyester or Dacron®) with aluminum or plastic shafts. Swabs with calcium alginate or cotton tips with wooden shafts are not acceptable.

Isolation of the RNA

Using QIAamp viral RNA extraction Kit Protocol for kit

Logspin: Protocol for spin columns

DIY RNA Extraction
DIY RNA Extraction No.2

Warnings and precautions

"RNA is extremely sensitive to RNases [...] Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination.". More info

RNase alert kit for detecting / debugging RNase. £191 for 25

Sample and size: proposal 1: saliva

Quick and easy to do. Ensure you are hydrated and then avoid drinking for 15 minutes (to ensure saliva is runny but not just water).

Spit up to 140 µl up to 500 µl into 1.5ml eppendorf.

Removal of cells

As the samples will contain cells: "Samples containing cells, such as [...] most swabs, should first be [...] centrifuged for 10 minutes at 1500 x g and the supernatant used.

Equipment needed

Centrifuge up to 1 ml at 1500g for removing cells Pipette tips (200 and 1000 ul) Pipette (200 and 1000 ul)

Preparation of reagents

Follow "Preparation of reagents" on page 17

Make "carrier RNA mix" from "Buffer AVE" and "carrier RNA", freezing rest as aliquots of _?_ ul

Calculate and make only amount of required "Buffer AVL-carrier RNA mix

For two samples this looks like: 1.12 ml 11.2ul

Buffer AW1

Requires 25ml ethanol

Buffer AW2

Requires 25ml ethanol

(Reverse Transcription) RNA to cDNA

Amplification via PCR

Paper uses "1 step RT-PCR mix" WELLS BIO INC., South Korea

ThermoFisher 25 for £286

Qiagen 200 for \$672

ThermoFisher 100 for £163

Load samples in following order:

- 1. primers
- 2. negative control 1
- 3. positive control
- 4. negative control 2

Required consumables & equipment

| Description | Comment | Number needed | Cost |
|---|---|------------------|------|
| Lab coat | | | |
| Gloves | To protect self from chemicals and avoid contaminating sample | | |
| Goggles | | | |
| | | | |
| RNase free Pipette tips (20, 200 and 1000 ul) | Preferably with aerosol barrier filters. Definitely need to be RNAase free sterile packs. If at home then no way to keep sterile. | 1 pack each | |
| Pipettes (20, 200 and 1000ul) | | 1 of each | |

| 1.5 ml eppendorfs | Sterile | | |
|--|--|---|----------|
| Microcentrifuge | With rotor for 1.5 ml and 2 ml tubes | | |
| 80°C water bath | if checked Buffer AVL for precipitate and it's present | | |
| Eppendorf rack | | | |
| | | | |
| 52904 QIAamp viral RNA extraction Kit for 50 RNA preps | Sample preparation. Storage of kit at 15 to 25°C | 1 | \$271.00 |
| Primers | See above for source | | |
| Ethanol (96–100%) | | | |
| ThermoFisher 100 for £163 | | 1 | £163 |

Sources of positive controls

https://eu.idtdna.com/pages/landing/coronavirus-research-reagents https://www.molecularcloud.org/How-to-detect-the-2019-novel-coronavirus.html

Related links

WHO page of protocols

https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance