Corona Detective User Protocol
(for the BBK Open Science Festival)

1) Sample collection and treatment
Collect about one mL of saliva (drool, not spit) in a sample tube.
In a fresh tube pipet 1µl of 100x Solution 1* and add 99µl of saliva to mix well.
Incubate 5 min at 95 ºC
(Can use a cup with boiling water, in resource-strapped settings)
Then, hold at room temperature until needed.
If solution seems cloudy, can pellet and use supernatant for reactions.

2) Rehydration of reaction pellets
Make sure dry reaction pellets are at bottom of tube and carefully remove sealing film from strip of tubes. (Detach with clean pipet tip and push down, if reactants are stuck to foil seal)
Pipet 10µl of 2x Solution 2* into each reaction tube, to resuspend pellets.

3) Isothermal amplification
Reactions will be incubated for 10 min at 55 °C and 45-60 min at 64 °C.
Many options for achieving stable incubation temperatures exist. Anything from a thermocycler, to precise heating blocks or even other options, like a sous-vide precision cooker, is possible.

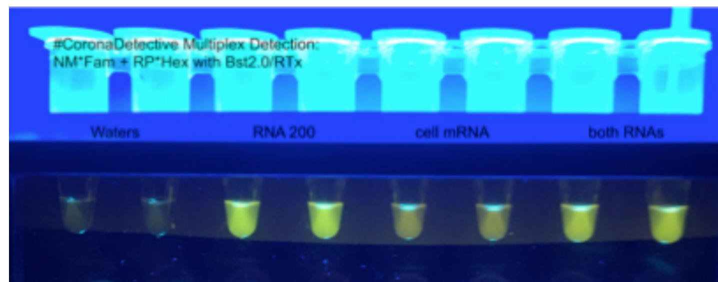
Set up the reactions.
Label tubes first, including at least one positive and one negative control.
(Corona Detective 8-tube strips can be produced so that they include these control samples already, as desired. In multiplex case, 5 tubes remain for samples.)
Pipet 10 µl water or 1x Solution 1 for the negative control; 9µl water + 1µl of the positive control(s); and 10 µl of treated samples to be tested into the remaining pre-labelled reaction tubes.
Carefully close the tubes, sealing well, with the provided strip of caps.
Mix Reactions Well. You can even vortex gently and either spin down or flick down, tapping gently.

Incubate the Corona Detective tubes for 10 min at 55 °C, to allow RTx to reverse transcribe the sample RNA to DNA, and then at 64 °C for at least 40 min (up to 90 min).

4) Detection
Cool tubes to Room temperature (or less) then place tubes in a fluorescence detector
(e.g. DIY GMO Detective Detector, gel transilluminator, or other) and take a picture.

Tubes positive for the presence of SARS CoV-2 N gene will exhibit bright green fluorescence.
The negative control (no template) should remain 'dark,' while designated positive viral control tube should exhibit bright green fluorescence. A weaker orange fluorescence will confirm the presence of human cell RNA, in the multiplex reactions especially if there was no virus RNA detected. This controls for extraction efficiency and should not be seen in negative control tubes.
If the positive control tube from the experiment does not exhibit bright green fluorescence, or the negative control does, the experiment has failed and needs to be repeated.

Example Multiplex Reactions:



Provided Solutions:

Solution 1 (100x TCEP/EDTA):
(from [Rabe and Cepko](https://www.pnas.org/content/117/39/24450): https://www.pnas.org/content/117/39/24450)
To make 5 mL, first, 358 mg of TCEP-HCl (Millipore Sigma 580567) is dissolved in water to create 2.5 mL of a 0.5 Molar (M) solution.
Then, 1 mL of 0.5 M EDTA, pH = 8 (ThermoFisher Scientific AM9260G) is added.
Finally, 575 µl of 10N NaOH is added and UltraPure water to bring the final volume to 5 mL.

Solution 2 (2x Rehydration Buffer):
2x Isothermal Amplification Buffer
10mM Magnesium Sulfate Solution

Reaction Pellet composition:
NM*FAM primer set (with RP*HEX primer set)
dNTPs
Warmstart Bst2.0 and RTx enzymes (NEB)
Trehalose
To note: Reaction Pellets should be protected from light and have a 'dry' appearance. If already sticky, some degradation of reaction efficiency is to be expected. Extra incubation time is possible, thanks to the sequence-specific 'quasr' (quenched fluorescence, https://doi.org/10.1021/acs.analchem.5b04054) detection. Complete details can be found in protocols.io, and a journal article is in press at the Journal of Biomolecular Techniques. (June2021)

Warning:

Never open the used tubes after reactions have been run!
To dispose of used tubes directly after pictures are saved is recommended.

For research use only, for surveillance screening (populations, including people without symptoms).

If a positive sample is observed (and controls look good), then the person should go for further official testing, and observe quarantine until results are obtained.

This protocol is being actively revised for ease of use, for instance, with blister pack liquid handing already being considered (to avoid pipetting)...

Your suggestions are welcome! (biolab@hackuarium.ch)