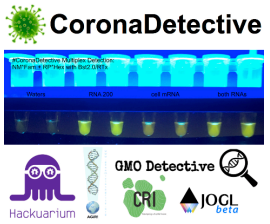
Nov 20, 2020 Version 2

# Corona Detective User Protocol V2.0 V.2

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**Protocol status:** Working
**We use this protocol and it's working**

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## Abstract

**Corona Detective** is based upon a molecular amplification strategy inspired by the '**GMO Detective**' method, in order to detect the virus causing Covid-19. Done not only without complicated equipment, but with a simple +/- fluorescent readout, the **Corona Detective** is very specific. Furthermore, controls to ensure sensitive detection, without false positives or negatives, are intrinsic to this solution. The final product, strips/plates of tubes with dry reagents, specific for Corona and a control gene (extraction control), can be shipped anywhere, without cold-chain dependence. Critically, monitoring tests could be run by ordinary people, by following this protocol. (Even kids have successfully participated in GMO detective workshops, also soldering their own fluor detectors.)

This user guide will allow you to test samples with this system, provided as freeze-dried reaction components in 0.2ml tubes made by this **protocol** (other formats possible e.g. 8-tube strips, 96-well plates, 1000 reactions, etc.).

**Human or clinical samples should only be run in settings with access to appropriate biosafety facilities, of course.**

Acknowledging the JOGL Open Covid 19 Initiative and all the #proj nucleic-acid-amplification team for support.

## Materials

MATERIALS

TCEP-HCl (Millipore Sigma 580567)
0.5 M EDTA, pH = 8 (ThermoFisher Scientific AM9260G)
2019-nCoV_N_Positive Control **Integrated DNA Technologies, Inc. (IDT) Catalog #**10006625

## Troubleshooting

## Safety warnings

**Before beginning to test any samples, environmental or clinicial, make sure to follow biosafety recommendations after appropriate risk assessment has been performed.**

**Ideally, keep 'post-reaction' detection area distinct from pre-reaction areas, with separate labcoats for each area, as already mentioned.**

**!! ** Do not open the reaction tubes after use, in order to avoid risk of contamination for future reactions. They can be simply disposed in the trash after pictures are taken. (This is recommended best practice.)**
**

## Before start

**Clean the working area, surfaces and pipettors with 10% bleach solution and then 70% alcohol.**
Use appropriate PPE, and change gloves and labcoats as needed.
When possible keep 'post-reaction' detection area distinct from pre-reaction areas, with separate labcoats for each area.

Safety information

***The products of isothermal amplification are extremely stable concatemers, that could readily cross-contaminate new reactions, if care is not taken from the beginning.***

Lab equipment (pipettors and tips, heating blocks, plate readers) is used in this protocol, but many alternatives exist, and the protocol can even be modified to just use simple droppers, environmental swabs, hot water and a DIY fluorescence detector (as in the **GMO Detective**, for 8-tube strips, or simple transilluminators for 96 well plates).

**The Corona Detective can be provided in several formats:**
**a) the simple 8-tube strip with the first 3 tubes for negative control, positive N and positive RNaseP controls, and thus allowing 5 tubes for sample tests;**
**b) the 96-well plate, again with 3 tubes devoted to appropriate controls, for the capacity to test 93 samples at once: and**
**c) other variants on the theme, +/- controls.**

**Rehydration buffer is provided with Corona Detective tubes, and consists of: (for one whole plate 96 reactions)**
200 µL 10X Isothermal Amplification Buffer +
100 µL Magnesium Sulfate (MgSO4) Solution +
1300 µL DNAse/RNAse free water ,
- ideally, all components should be stored until use in a cool dark place or in a fridge ( 4 °C ), but they are stable for shipment.

1 **Sample Extraction:** 5m

Two options are possible
A: Direct reactions of inactivated sample (i.e. 100 µL i.e. saliva, with the addition of 1/100 100x TCEP/EDTA inactivation reagent, and after incubation at 95 °C for 00:05:00 ) as in **Rabe and Cepko**

B: RNA Concentration with 'Magnetic Beads' or other validated extraction methods as **described** or **here**.

The B method for Sample Extraction may be especially for highly sensitive detection of SARS CoV-2 virus (N gene target), but the A method is simpler, and very sensitive, (and may be useful also for certain scenarios, like surveillance screening: especially if one would prefer to identify only people who might most be expected to currently transmit virus).

Extraction/concentration methods (B) take longer and are more expensive in addition to requiring a bit more infrastructure and careful elution to add the sample to Corona Detective components but can improve sensitivity (especially if pooling samples for testing).

**100x TCEP/EDTA** (from **Rabe and Cepko**)
To make 5 mL of 100× inactivation reagent, first, 358 mg of TCEP-HCl (Millipore Sigma 580567) was dissolved in water to create 2.5 mL of a [M] 0.5 Molarity (M) solution. Then 1 mL of 0.5 M EDTA, pH = 8 (ThermoFisher Scientific AM9260G) was added. Finally, 575 µL of 10N NaOH is added and UltraPure water to bring the final volume to 5 mL .

- 4 µL of inactivated A or eluted B sample is used in each 20 µL CoronaDetective reaction

2 **Isothermal amplification** 3h 15m

Set up the water bath or other incubators, in order to incubate reactions 00:10:00 at 55 °C and 00:45:00 at 64 °C Celsius.

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.

Set up the reactions.
Label carefully, including at least one positive and negative control.
(Corona Detective 8-tube strips can be produced so that they include these control samples already.)

Make sure lyophilized pellets in the tubes are at the bottom of all the tubes before carefully opening them. Open the tube containing rehydration buffer and add 16 µL Rehydration buffer (provided with Corona Detective) into each of the reaction tubes.

(For the 96 well format, a multipipettor or dispensing pipette is useful for this step).

Add 4 µL of clean water to designated negative control tube/s (closing lids as you go when possible).
Add 4 µL of clean water to designated Positive control tube/s, with included freeze-dried DNA control (or add 4 µL your own control solution, for instance, RNA controls, which are not expected to survive the lyophilization. )
Then, add 4 µL of the test samples (from step **1)** to the rest of the test tubes.

Safety information

MAKE SURE ALL WELLS ARE SEALED WITH APPROPRIATE CAPS OR ADHESIVE SEALING FILM!

Mix Reactions Well. You can even vortex gently and either spin down or flick down, tapping gently.

Incubate the Corona Detective tubes for 00:10:00 at 55 °C, to allow RTx to reverse transcribe the sample RNA to DNA.

Incubate the Corona Detective tubes at 64 °C for at least 00:40:00 (to 01:30:00 ) and cool to Room temperature for Detection (step 3).

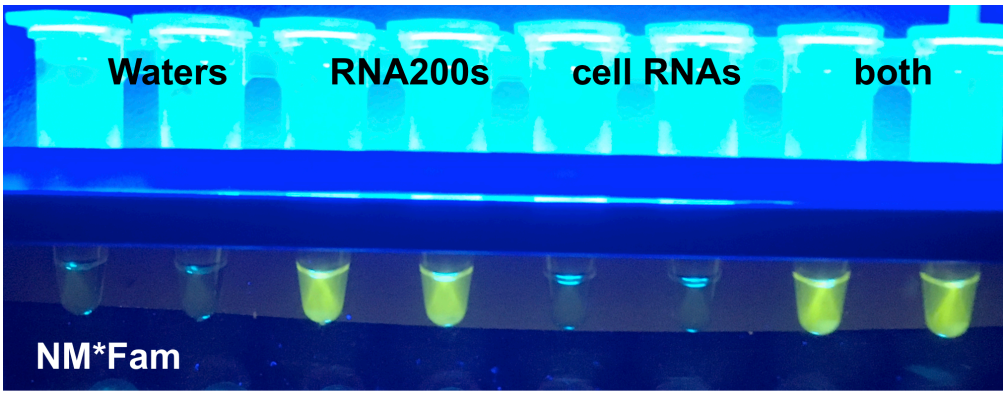
If not reading results right away, tubes can be placed in a fridge 4 °C . The results are stable for weeks even at Room temperature .

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

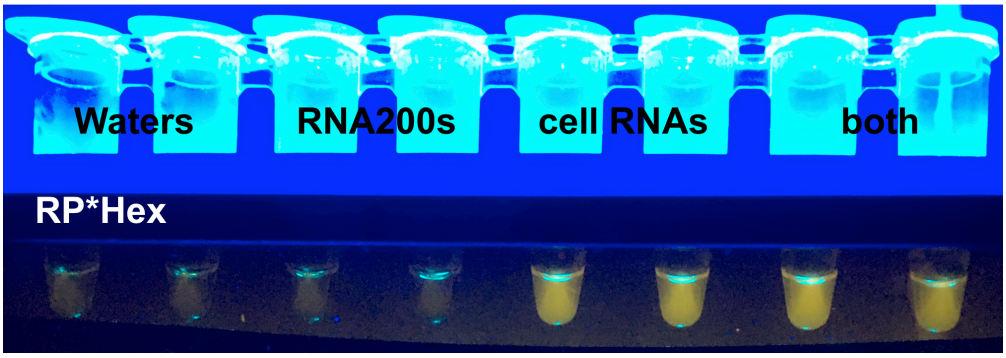
Tubes positive for the presence of SARS CoV-2 N gene will exhibit bright green fluorescence.
The negative control (no template) should remain 'dark,' while designated positive viral control tube should exhibit bright green fluorescence.
A weaker orange fluorescence will confirm the presence of human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

QUASR fluorescent signal develops as the short quencher primers are displaced through amplification of product, with the N-target labeled with the FAM fluorophore, a blue LED excitation filter and amber emission filter are ideal (as in the GMO detective system).
The internal control for RNAseP is labeled with the HEX fluorophore, which can also be seen (more orange, less green) with the DIY Detector from GMO detective.

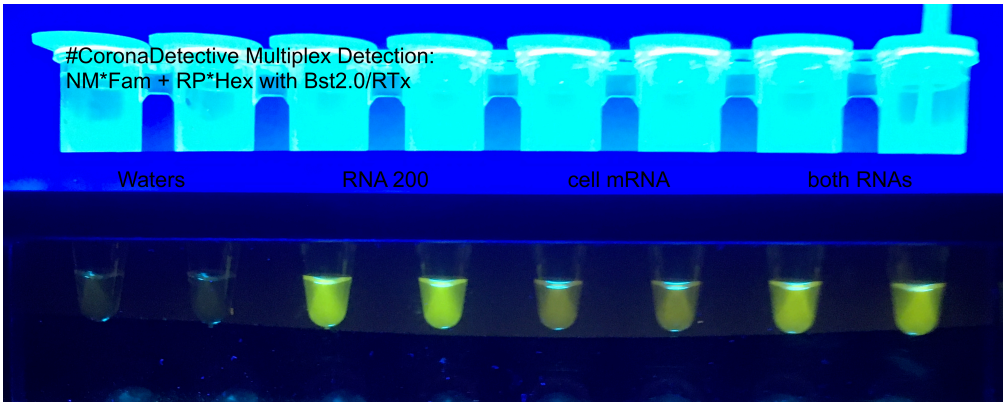
Single primer set reactions and then the multiplex reactions are shown below as examples:




Example of single reactions with QUASR Detection in Detector




Examples of single primer sets with the QUASR detection, to illustrate the difference between the FAM
and the HEX signals in the ordinary GMO Detective Detector. 200 copies of the synthetic BEI RNA per
20ul reaction result in very bright green fluorescence, while the RNAseP (internal extraction control) gives
a more orange signal.




Control results for the Corona Detective reactions (multiplex with both sets of primers).

***Removing the blue filter and including a more orange emission filter, allows these two signals to be distinguished, even on a cell phone camera (an old iPhone SE was used above). However, red (tagging alternatively the RNAseP primer with a TexasRed***

***fluorophore) may also be used to distinguish the two signals better, with the same blue (or green) excitation and a red emission filter.***

Avoiding saturating the camera sensor is important, to make sure you can distinguish negative from positive results. (Auto-adjusting exposure, if not automatic, can usually be initiated with a tap on a bright point of the image.)

Old sample tubes from this reaction, if protected from light and simply kept at room temperature, can be looked at weeks later with no obvious change in the resulting fluorescence.

Safety information

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

***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.***