COVID19 homemade test

0. Nasopharyngeal swab (NPS) (infectious!, use personal protection equipment):
Health care systems should have in place procedures to take nasopharyngeal swabs by trained staff, but if you need to do it yourself, there are a couple of excellent sources to learn the procedure:

- https://www.youtube.com/watch?v=DVJNWefmHjE
- https://www.ottawapublichealth.ca/en/professionals-and-partners/how-to-collect-a-nasopharyngeal--np--swab.aspx

* One big advantage of setting up the NPS collection near or at the testing lab is that you can avoid using transportation medium and the problems associated to the transportation of infectious samples. You only need **sterile swabs**, much cheaper and without supply problems, compared to the transportation medium for viral samples. The sample goes **from the nose to the lysis buffer**, minimizing exposure.
** Organize yourself before starting: ideally, people ID or email should be entered already and associated to a bar-code that you can stick to tubes with 750ul 0.2% SDS-10 mM Tris HCl pH8.

1. Sample manipulation (infectious!):
Sample manipulation and LYSIS (ideally under the hood, all-gear protection) for fresh NPS (taken next to the hood):

1. Prepare tubes with 750 ul 0.2% SDS-10 mM Tris HCl pH8
2. Place the swab into the tube and mix well; if you're taking many samples, go to the next and leave the NPS sitting in lysis buffer for a while (for instance, do batches of 16 people - it takes less than a minute to take one sample-). Discard the swab (infectious trash bin, although it's not infective after SDS exposure); you end up with about 500 ul lysis buffer
3. Add 20 ul 10 mg/ml proteinase K
4. Incubate at 65ºC x 15 min.

# *Alternative Sample manipulation* and LYSIS (under the hood, all-gear protection) for transported NPS (taken away from the lab and transported in medium such as M medium*):

1. Prepare plate with 5ul 10 mg/ml proteinase K per well
2. Vortex 30sec. tubes with swabs, spin briefly and take 50ul of medium into the prot.K wells
3. Incubate at 65ºC x 15 min.

* It's a bad idea to use special medium designed to keep viruses alive for the purpose of screening for coronavirus by rt-PCR; but for whatever reason, the use of M medium for NSP collection has been the standard in the current outbreak.

2. RNA preparation (96 well plates, magnetic plate, best with side-magnet such as DynaMag-96 side) #:

1. Add (per well):
    a. 150ul binding buffer
    b. 5ul magnetic beads
    c. 50ul lysate (if A protocol*)

* *if B protocol*: use the same lysis plate and add to each well 150ul binding and 5ul beads

# There're several vendors for RNA purification with magnetic beads, they all work similarly
If you don't have a magnetic plate, you can put together one just using parts from a pipette tip box and toy magnets (such as Maxmag bars) or lab magnetic bars of the right size:

2. Tape and shake at room temp. 2000 rpm x 5min. If you don't have a shaker, just shake it by hand once in a while for 5 minutes.
3. Spin down plate to clear tape and prevent cross-well contamination; remove tape and place plate on magnet
4. Working with multichannel and filter tips, aspirate and discard liquid*

* the nice thing about side-magnets is that you can bring down the tips, straight to the bottom, without concern to touch the mag.beads

5. Take plate off magnet and add 100ul wash 1; pipette up-down a couple of times*

* with multichannel, you'll repeat these steps several times along the procedure: off-magnet: add buffer to one column of wells, and with the same tips, pipette up-down to wash, next column... until the whole plate has been washed on-magnet: aspirate buffer from all the wells

6. & 7 repeat wash with wash 2 and wash 3; make sure you remove all the liquid after wash 3
7. Let the beads dry at room temp. 5min.
8. Add 20ul eluting buffer (or RNA-water) and incubate at 70ºC 10 min. (you can use a PCR machine
9. On-magnet, aspirate 20ul eluate and keep in a new plate or just aspirate 10ul for a test and leave the rest on the original plate, in case you need it for confirmation test. You have RNA for 2 tests (10ul each).

3. RT-PCR (protocol from Virology-Charité Hosp., Berlin: https://virologie-ccm.charite.de/en/)
In the current outbreak, you can assume that the primers for gene E-Sarbeco, although not specific for SARS-CoV2, are detecting the coronavirus responsible for COVID-19. If you want to be more specific, you can confirm with a secondary test specific for SARS-CoV2 such as RdRp (for more information visit https://virologie-ccm.charite.de/en/ or other reputable sources: WHO, CDC...)

| Per sample: |
| --- |
| 13 ul 2X RT-PCR buffer + dNTPs mix (invitrogen, superscript III system, for instance)<br>0.4 ul 50 mM MgSO4<br>1 ul non-acetylated BSA (1mg/ml)<br>1 ul E-Sarbeco primers+probe (forward and reverse at 10 uM, probe at 5uM)<br>1 ul RT+Taq mix<br>+ 10 ul sample RNA (typically at 4-8 ng/ul, but it varies a lot depending on swab procedure and patient condition) |

| PCR profile: |
| --- |
| 55 C 10min<br>94 C 3 min<br>[94 C 15 sec; 58 C 30 sec; read fluor.] x 45 cycles |
| Primers and Probe |
| E_Sarbeco_F1: ACAGGTACGTTAATAGTTAATAGCGT<br>E_Sarbeco_R2: ATATTGCAGCAGTACGCACACA<br>E_Sarbeco_P1: FAM-ACACTAGCCATCCTTACTGCGCTTCG-BHQ1 |