

Figure 2. The sponge in the tube and when removed from the tube. Keep the cap clean. After oral fluid has been collected the sponge is put back in the tube in reverse order.

The oral fluid is extracted from the sponge using a centrifuge. Procedure 1) Remove the cap and remove the sponge from the tube and insert the sponge in the cap (Figure 3). Place the cap with sponge attached back on the tube (Figure 3). Centrifuge for 10 minutes 3,000 rpm in a table top centrifuge at room temperature, preferable with closed buckets. Remove the cap with sponge and discard as contaminated material. Remove the oral fluid used a pipette and aliquot as appropriate. Store at -80°C until use.

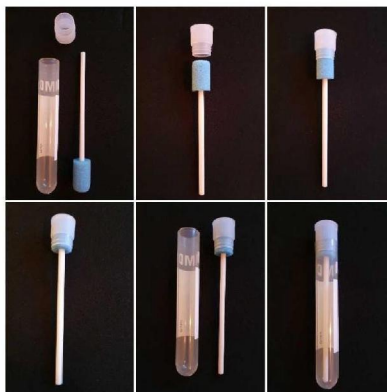


Figure 3. Sponge with oral fluid removed from the tube, inverted and sponge inserted in the cap and next the cap with sponge inserted in the tube for centrifugation.

Procedure 2) Alternatively, after opening the collection tube, with tweezers take the sponge, cut 3 cm from the shaft using scissors, invert the sponge and insert in the tube upside down. Close the

tube with the cap and centrifuge for 8 minutes 800 g in a table top centrifuge at room temperature, preferable with closed buckets. Remove the cap and take the sponge out with tweezers and discard as contaminated material. Remove the oral fluid used a pipette and aliquot as appropriate. Store at -80°C until use.

For molecular detection we use Roche COBAS4800 with CT/NG kit extraction or Roche MagNAPure extraction and in-house implementation of E-gene and RdRP-gene Corman et al. real-time RT-PCR on LC480 II using fast virus master mix chemistry [5]. For COBAS4800 extraction, 300 µl specimen is mixed with 300 µl CT/NG lysis buffer and 25 µl Equine Arteritis Virus (EAV) internal control; 400 µl is used for extraction and eluted in 100 µl. 10 µl is used in the PCR. Routinely we mix 200 µl specimen with 275 µl MagNAPure blue extraction buffer with EAV internal control and yeast tRNA included; 450 µl is used for extraction and eluted in 50 µl. 5 µl is used in the PCR. If there is not enough oral fluid the volume is supplemented with DNase/RNase free physiological salt solution. However, in practice there is usually enough volume oral fluid.

A pilot was conducted using COBAS4800 extraction. Preliminary results of young children (n=3; median age 13 range 13-15 years) and adults (n=10; median age 47 range 40-47 years) show that there is good concordance between oral fluid (OF) and nasopharyngeal (NP) swab and OF and oropharyngeal (OP) swab (Figure 4). The Ct values are however slightly to considerable higher in OF compared to those in NP and OP swabs (Figure 5). That might have resulted in negative OF result if the load in NP and OP was already very low.

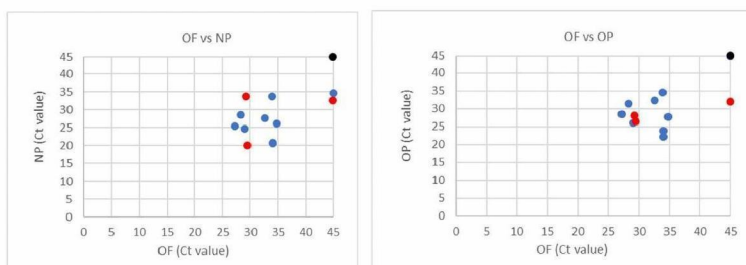
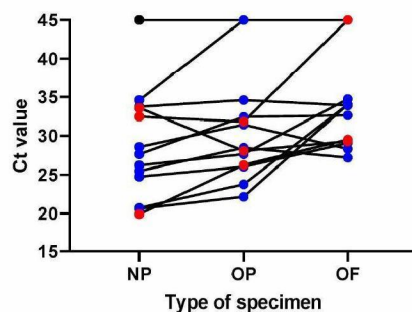


Figure 4. Comparison of Ct values for E-gene SARS-CoV-2 RT-PCR in oral fluid (OF) versus nasopharyngeal (NP) and oropharyngeal (OP) swab. Ct 45 = negative in PCR. For one patient (black dot) OF, NP and OP were negative, but the feces was positive in PCR for SARS-CoV-2. Children labeled with red dot, adults with blue or black dot.



lower respiratory specimens. Sometimes with slightly higher and sometimes with slightly lower SARS-CoV-2 detection rates compared to NP swab.

In conclusion, taken into account recently published work and our preliminary findings, collection of OF instead of NP and OP swabs is a good alternative for SARS-CoV-2 detection in the upper respiratory tract.

References

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