

Brief summary of using oral fluid specimens for detection of SARS-CoV-2 infection in cases suspect for COVID-19

(10)(2e), (10)(2e), (10)(2e), (10)(2e), (10)(2e), (10)(2e), (10)(2e)
(10)(2e), (10)(2e), on behalf of the first few hundred study group

Centre for Infectious Disease Control, National Institute for Public Health and the Environment,
Bilthoven, The Netherlands. Contact: (10)(2e) (10)(2e)@rivm.nl

FFX COVID19 study The Netherlands

Most frequently used specimens for SARS-CoV-2 detection are nasopharyngeal (NP) (or mid turbinate nasal) and oropharyngeal (OP) swabs, with NP (or midturbinate) in general more frequently and for a longer period positive than OP. Because of shortages of NP swabs, alternatives are sought. In The Netherlands for many years in the surveillance of mumps, measles and rubella and pneumococci oral fluid is being used for molecular detection of the virus or bacterium [1-4]. In the first few hundred COVID-19 cases study in the Netherlands with special attention for the involvement of young children in the transmission of SARS-CoV-2, oral fluid has been collected primarily for IgA detection and secondarily for studying the possibility of virus detection and contribution to transmission.

Oral fluid is collected by a simple device Oracol S10, Malvern Medical Developments (<https://www.malmed.co.uk/saliva-collection/>) (Figure 1).



Figure 1. Oral fluid collection system Oracol S10.

The procedure for use is simple. The device is designed to be used in a similar way to a toothbrush. Remove the cap from the tube, take out the sponge (Figure 2). Oral fluid is collected by rubbing the sponge swab firmly along the gum (at the base of the teeth if present) at both sides of the mouth until the sponge is wet; this takes about 1 minute. For SARS-CoV-2 investigation we use 2 minutes to be absolutely sure there is enough oral fluid collected. Once the sponge is sufficiently wet, replace the sponge back into the test tube and insert the cap (Figure 2 in reverse order).



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. 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, 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.

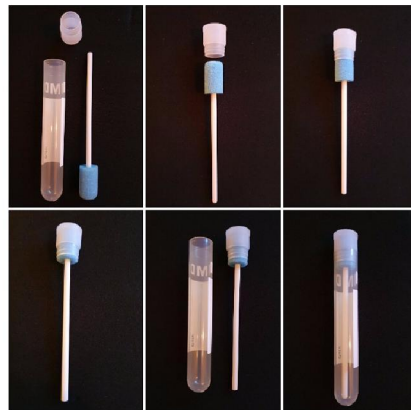


Figure 3. Sponge with oral fluid removed from the tube, inverted and sponge inserted in the cap and next the cap with sponge inserted in the tube for centrifugation.

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 μ l. 10 μ l is used in the PCR. Routinely we mix 200 μ l specimen with 275 μ l MagNApure blue extraction buffer with EAV internal control and yeast tRNA included; 450 μ l is used for extraction and eluted in 50 μ l. 5 μ l 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) and adults (n=9) 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.

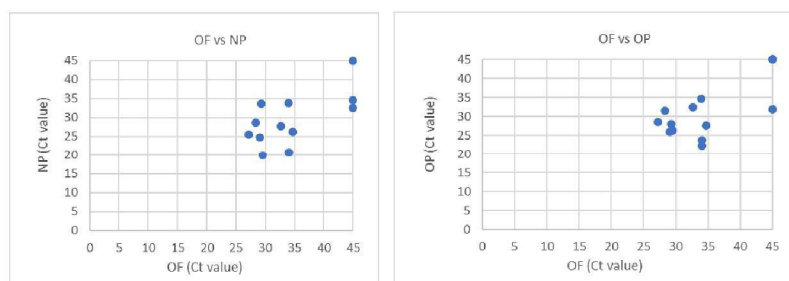


Figure 4. Comparison of Ct values for E-gene SARS-CoV-2 RT-PCR in oral fluid (OF) versus nasopharyngeal (NP) and oropharyngeal (OP) swab. Ct 45 = negative in PCR. For one patient OF, NP and OP were negative, but the feces was positive in PCR for SARS-CoV-2.

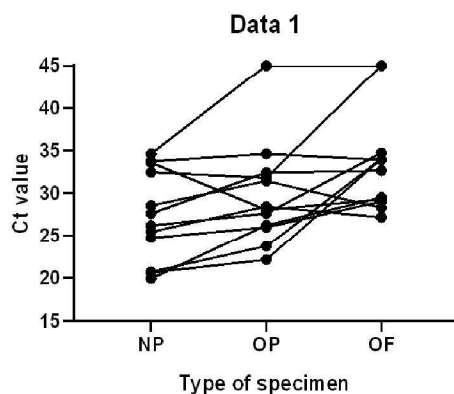


Figure 5. Relationship of SARS-CoV-2 E-gene RT-PCR Ct values in nasopharyngeal swab (NP) with that in paired oropharyngeal swab (OP) and oral fluid (OF). Ct 45 = negative in PCR. The patient negative for NP, OP and OF not shown.

Literature summary (with special thanks to (10)(2e) and (10)(2e))

Self-collected deep throat saliva (posterior oropharyngeal saliva) was suggested as alternative to sputum and yielded positive PCR results in 11 out of 12 hospitalized COVID-19 patients in Hong Kong, as well as 3 positive and 2 negative virus cultures [6]. Further cohort of 23 patients with 173 deep throat saliva or endotracheal aspirate specimens studied by the same group found median viral loads of 5.2 log₁₀ copies per mL (IQR 4.1–7.0) at presentation. The highest saliva viral loads were reported in the first week since symptoms onset for 20 patients, followed by gradual decline and prolonged detection of 20 days or more in 7 patients [7]. A pre-print study in the USA including 44 cases reported comparable or superior sensitivity of saliva to nasopharyngeal swabs and higher SARS-CoV-2 titers in saliva for 38 matched specimens [8]. A study in Italy analyzed saliva specimens of 25 confirmed COVID-19 patients and all were found PCR positive whilst two patients showed positive salivary results on the same days when their pharyngeal or respiratory swabs showed conversion to negative [9]. They reported the later two cases in more detail separately raising the concern of possible transmission when saliva is positive and upper respiratory specimens negative [10]. A study in Zhejiang, China confirmed SARS-CoV-2 infection in 96 patients by testing 668 sputum and 1178 saliva samples but did not specify positivity rates the samples types separately. Taken together the positivity rates declined from 95 to 54% in the first 4 weeks since symptoms onset with a median virus shedding duration of 18 (IQR 13-29) days [11]. Fang and colleagues reported SARS-CoV-2 detection in saliva for 25 cases for a period of 13.33±5.27 days in mild cases and 16.50±6.19 days in ICU patients [12]. A study in Australia analyzed 522 paired saliva and nasopharyngeal swabs of COVID-19 suspect cases; 39 had positive nasopharyngeal swab of which 33 also positive saliva. Viral loads were lower in saliva compared to nasopharyngeal swab with both positive up to 21 days post symptom onset. Among 50 PCR-negative patients one had a positive saliva specimen [13].

A remark has to be made on the type of specimen reported as saliva. In the above studies it ranged from posterior oropharyngeal saliva collected by spitting or using the drooling technique collecting oral fluid after 1 to 2 minutes waiting and collecting it using a pipette or a sponge how we did it. Nevertheless, all techniques resulted in reliable detection of SARS-CoV-2 compared to upper or lower respiratory specimens.

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.

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