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

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[redacted], on behalf of the first few hundred study group

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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 tub, 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. The collected specimen is transported cooled in a coolbox with ice packs to the lab where the specimen is processed at arrival.

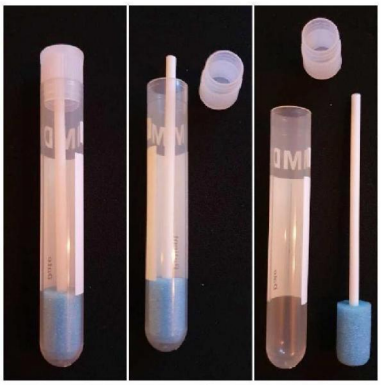

Figure 2. The sponge in the tube and when removed from the tube. Keep the cap clean. After oral fluid has been collected the sponge is put back in the tube in reverse order.

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.

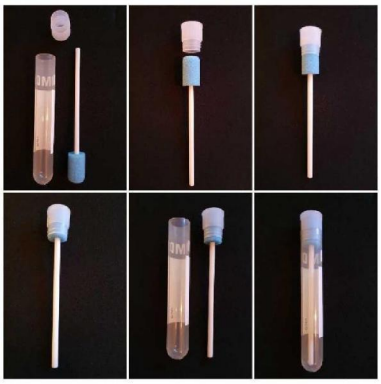

Figure 3. Sponge with oral fluid removed from the tube, inverted and sponge inserted in the cap and next the cap with sponge inserted in the tube for centrifugation.

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 or MagNApure extraction of oral fluid from 17 adults and 28 children. Two adults and 7 children were selected being negative in PCR for nasopharyngeal (NP) and oropharyngeal (OP) swab originating from families with members having COVID-19; oral fluid (OF) of all persons was negative in PCR for SARS-CoV-2. Twenty-one children (median age 12 range 2-16 years) and 15 adults (median age 46 range 18-61 years) were selected being positive in PCR for NP and/or OP swabs. For the 15 adults there is good concordance between OF and NP swab and OF and OP swab (Figure 4). The Ct values are however slightly to considerable higher in OF compared to those in NP and OP swabs (Figure 4). Nevertheless, only one patient who had a positive OP swab and negative NP swab, indicative for low viral load, was negative for OF.

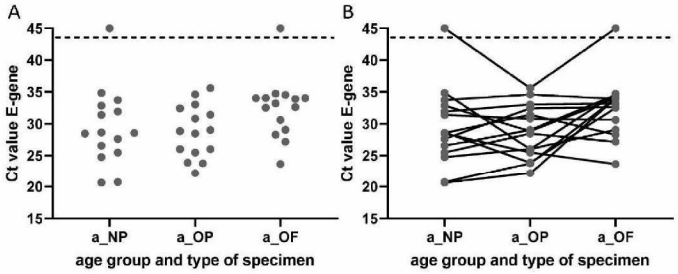

Figure 4. Comparison of Ct values for E-gene SARS-CoV-2 RT-PCR in oral fluid (OF) versus nasopharyngeal (NP) and oropharyngeal (OP) swab for adults (a). A. Distribution of Ct values by specimen type. B. Ct values for all specimen types connected by patient. Ct 45 = negative in PCR. For one patient only OP was positive.

For the 21 children the results were different (Figure 5). In total, of 13/21 (62%) children the OF specimen was positive. However, similar to the single adult, of 5/6 children with only one of NP or OP swabs being positive the OF specimen was negative. Of a further three children of whom the OF specimen was negative the NP and OP specimens had Ct values larger than approximately 29.

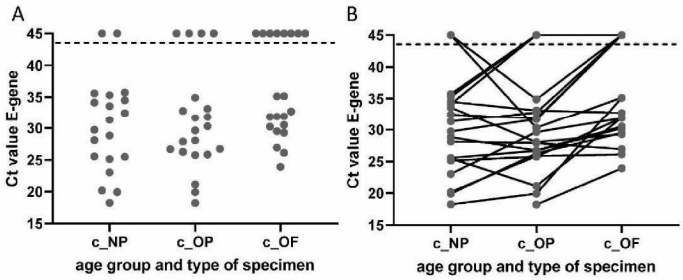

Figure 5. Relationship of SARS-CoV-2 E-gene RT-PCR Ct values in oral fluid (OF) versus nasopharyngeal (NP) and oropharyngeal (OP) swab for children (c). A. Distribution of Ct values by specimen type. B. Ct values for all specimen types connected by patient. Ct 45 = negative in PCR.

Of the 13 patients with positive OF specimen this specimen type had frequently a higher Ct compared to NP and OP swab (Figure 5B).

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 NP 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 tract 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 for 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 NP swabs of COVID-19 suspect cases; 39 had positive NP swab of which 33 also positive saliva. Viral loads were lower in saliva compared to NP swab with both positive up to 21 days post symptom onset. Among 50 NP 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. 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. However, patients with low viral load NP and/or OP specimens will be missed when OF is collected alone.

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