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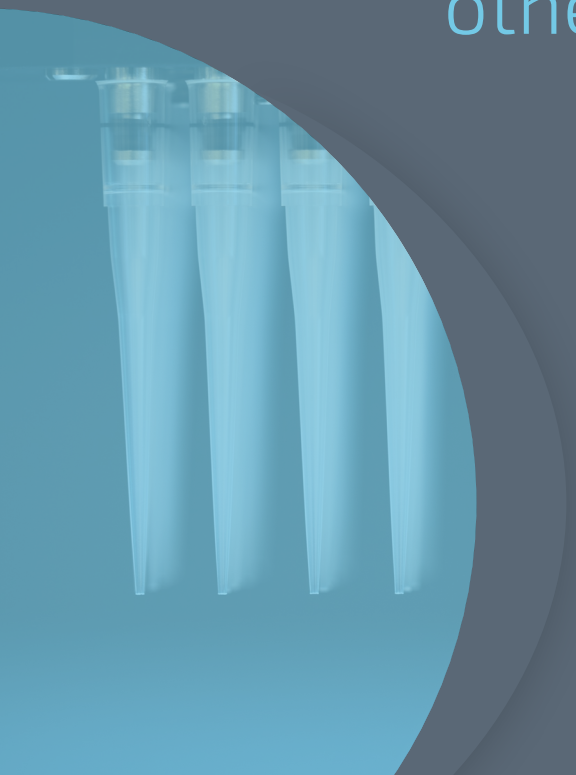
SARS-CoV-2 PCR saliva test

Preventive screening through **repetitive SARS-CoV-2 PCR testing on saliva**

in companies and other organizations



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Background

There is no perfect strategy to prevent SARS-CoV-2 outbreaks at places where people come together (like in schools, companies, nursery homes, …) except for a complete lockdown. A thorough testing and screening strategy is therefore crucial to detect, as early as possible, persons who are potentially infectious, followed by isolation. It is obvious that, for preventive screening, a repetitive testing strategy with a less sensitive method is preferable over a one-time testing strategy with a more sensitive nasopharyngeal (NP) RT-PCR1-3.

Asymptomatic screening for SARS-CoV-2 is currently performed using either saliva PCR testing or rapid antigen testing. Saliva PCR testing has the advantage of easy self-sampling and therefore does not require the intervention of a healthcare worker. Moreover, sampling with a nasopharyngeal swab for rapid antigen testing results in significant discomfort, and is as such not desirable for repetitive testing. On the other hand, saliva PCR testing needs transport to a laboratory and test results take several hours while the rapid antigen test has almost immediate test results. The cost of saliva PCR testing is significantly lower than rapid antigen testing, mainly due to sampling not requiring a healthcare professional.

nasopharyngeal RT-PCR	saliva RT-PCR	rapid antigen test
intervention by healthcare worker needed	self-collection, no healthcare worker needed	intervention by healthcare worker needed
high sensitivity	medium sensitivity	medium sensitivity
invasive, sometimes painful sampling	pain-free sampling	invasive, sometimes painful sampling
not suitable for repetitive testing	suitable for repetitive testing	not suitable for repetitive testing
24 hr until result	24 hr until result	result within 30 min
high cost	**low cost**	**medium cost**

Table 1: Comparison of different SARS-CoV-2 tests

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National SARS-CoV-2 saliva study4

During the spring and summer of 2020, a total of 3000 individuals (both symptomatic and asymptomatic) at Belgian triage centers participated in a study where individuals were sampled using three procedures: (1) a classical nasopharyngeal swab, (2) a saliva spitting sample and (3) an oral swabbing sample. The same PCR test for SARS-CoV-2 was performed on all three samples.

Considering the nasopharyngeal swab as the reference test, it was found that saliva spitting samples gave superior results compared to the oral swabbing samples. All infected individuals (symptomatic and asymptomatic) with a nasopharyngeal swab Cq value of <24.5 (equivalent to 1.5 million copies/ml) presented a positive saliva spitting sample. These results indicate that both symptomatic and asymptomatic individuals with virus levels considered as infectious (moderate to high levels)5 are detected with excellent sensitivity using a saliva PCR test.

Biogazelle pilot study

A pilot study for preventive screening of asymptomatic individuals using saliva PCR testing was started in January 2021. It was supported by an online and anonymous sample registration and result communication webtool. The study aimed to assess participant compliance, suitability of the sampling kits and webtool for registration, logistics for centralized collection and transport from the companies/organizations to the laboratory, and the analytical part of the test. During the pilot, the overall process was further fine-tuned, and the speed of analysis and reporting was evaluated.

Five companies, three nursing homes and two schools, with a total of 350 participants provided morning saliva samples twice a week during a period of four weeks. A total of 2158 samples were examined. All samples were found negative except for one sample. This sample came from an employee who had COVID-19 symptoms 3 weeks prior to the saliva sampling, stayed in quarantine, came back to work and tested positive in the pilot study. This is not an uncommon observation since PCR positivity of viral fragments is reported up to 30 days or more after symptoms.

A questionnaire was sent out to the participants after completion of the pilot study. Ten percent indicated difficulties using the anonymous online registration process. To address this issue, we optimized the registration process by providing a personal QR-card to each participant simplifying the online registration process. Ninety percent of the participants indicated they experienced no problems during the whole pilot study. The motivation for the testing was very high among the participants (score of 4.8/5), and many indicated they found it important that companies or organizations would subscribe to a saliva testing program (score of 4.6/5).

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Saliva SARS-CoV-2 PCR testing method validation

Saliva was collected using Saliva RNA Collection and Preservation Devices (Norgen Biotek, #53800) containing a preservation buffer. Sample transport was performed at room temperature. Upon arrival in the laboratory, the tube caps were heat inactivated for 40 minutes at 80 °C in an oven. In the National SARS-CoV-2 saliva study, RNA extraction was performed using the Total RNA Purification Kit (Norgen Biotek #24300) on individual samples. In the Biogazelle pilot study, three samples were pooled (33 µl each) using a Tecan Freedom EVO 200 liquid handler, followed by MagSI-NA Pathogens RNA extraction (Magtivio #MDKT00210960) and eluted in 75 µl. Six µl of RNA eluate was used as input for a 20 µl duplex RT-qPCR reaction in a Bio-Rad CFX384 qPCR instrument using 10 µl iTaq Universal Probes One-Step RT-qPCR mastermix (Bio-Rad # 1725141) according to the manufacturer's instructions, and 250 nM final concentration of primers and 400 nM of hydrolysis probe. Primers and probes were synthesized by Integrated DNA Technologies using cleanroom GMP production. For detection of the SARS-CoV-2 virus, the Charité E-gene primers/probe were used (FAM channel); for the internal spike-in control, a proprietary hydrolysis probe assay (HEX channel) was used. Cq values were generated using the FastFinder software v3.300.5 (UgenTec).

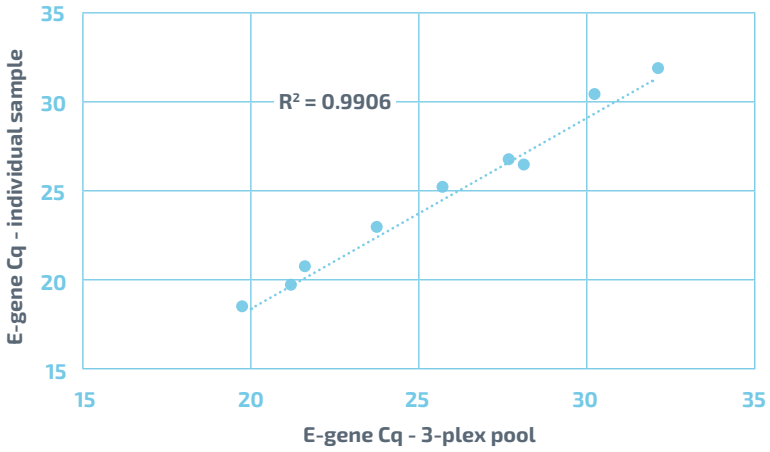

Figure 1: Excellent concordance between individual sample Cq value and result from 3-plex pool, with lower Cq values (as expected, i.e. higher sensitivity) for the individual (unpooled) sampletests

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During the validation of the MagSI-NA RNA extraction, no effect of heat inactivation on the Cq value was observed. The effect of pooling of three samples on the signal (Cq value) was assessed and resulted in a mean delta-Cq of 0.94, which is significantly lower than the 1.58 expected from the 1:3 dilution ratio. The limit of detection (LOD95) of the pooled PCR saliva test was determined through spiking of Armored RNA Quant SARS-CoV-2 Panel (Asuragen #52036) and was found to be 4000 copies/ml per individual sample. Pooling did slightly decrease the repeatability of the assay (standard deviation of Cq value of 0.85 compared to 0.23 for the unpooled samples) but remains within the acceptable range of 1 Cq (corresponding to a 2-fold difference). No significant change of Cq values was observed after saliva samples were stored 7 days at room temperature.

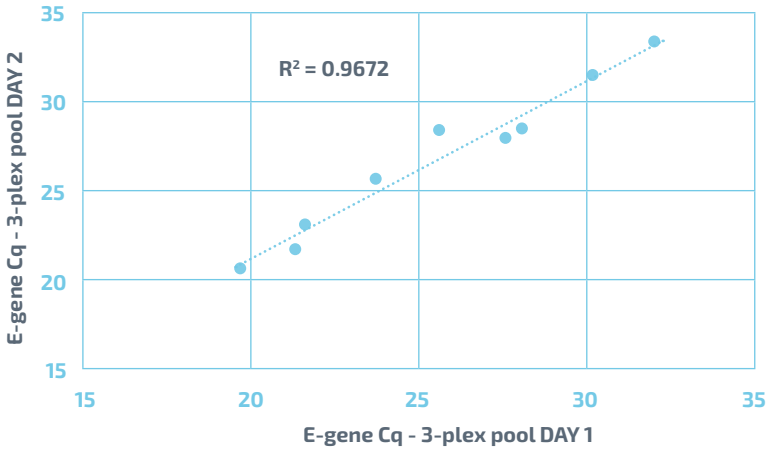

Figure 2: Excellent reproducibility of the complete workflow (pooling, RNA extraction and RT-qPCR) over a wide dynamic range

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