

# Saliva stabilization





Swab preservation InActiv Blue® is dedicated in providing high quality solutions for safe transport of infected patient samples, including SARS-CoV-2



Product code: REF IB\_TUB



Product code: REF IB\_CD01







### About the medium

### InActiv Blue®

is a CE-IVD marked, virus inactivating and RNA stabilizing transport medium for molecular analyses including RT-qPCR.

The medium is perfect for preservation of swabs and saliva before testing.

#### **Key features**

- Perfect for preservation of nasal/throat/nasopharyngeal swabs and saliva
- Complete inactivation of SARS-CoV-2 within 1 minute; also effective inactivation of vaccinia virus, norovirus, parvovirus, ...
- Safe and stable preservation of RNA up to 30 days at room temperature
- ♦ No cold chain required
- Slue dye as visual pipetting control
- N Independent validation with a range of common RT-qPCR platforms
- CE-IVD marked
- Mucolytic properties to reduce viscosity in mucus rich samples
- Each batch produced and tested according to the highest quality standards in ISO certified labs

#### **Quality control**

- SARS-CoV-2 RNA stability test
- Virus inactivation test



InActiv Blue®

**REF IB\_TUB** 

Further reading Performance details

p 11

(1x PP tube with 2 ml virus inactivating and RNA stabilizing transport medium)



## Blue Collect™

Innovative CE-IVD marked saliva collector with volume control (1.3 ml). Validated for SARS-CoV-2 detection.

#### **Key features**

- Simple device to collect saliva, also for children (+5 years)
- Convenient design for self sampling
- Easy visualisation of the required saliva volume due to transparent collector
- N Inert material: no inhibition of RT-qPCR
- Ideal for large scale screening purposes
- 🔨 Only small volume of 1.3 ml of saliva needed
- When used in conjunction with InActiv Blue<sup>®</sup> containing tubes, viscosity of saliva is greatly reduced

#### **ROBUST SAMPLE STABILITY AT 37 °C FOR 1 WEEK**

InActiv Blue<sup>®</sup> tubes and DNA/RNA Defend<sup>™</sup> are perfectly suitable to stabilize **saliva** over a period of at least 8 days, even when the volume deviates from the recommended 1.3 ml. This is confirmed by an additional small study whereby different volumes of saliva (0.5 - 0.65 - 1.3 - 2.45 ml) of two patients that were diagnosed with COVID-19 by PCR, were added to 2 ml InActiv Blue<sup>®</sup>. Samples were sent to a certified medical lab for RT-qPCR on day 1. After analysis, tubes were stored at **37** °C (worst case storage condition) and retested on **day 8**. Cq values do not differ between conditions and over time, which reconfirms the robustness of InActiv Blue<sup>®</sup> and DNA/RNA Defend<sup>™</sup> as virus transport medium for saliva.



If possible: 🔇 🔇 🧐	0 min before collection	
The second secon	Wash your hands ith soap and water. 2 Blue Collect <sup>™</sup>	
Hold the collection device (Blue Collect™) as shown in the picture.		Close and shake gently.
Brin	ng towards your lips.	
ill until the lower part is full, not including foam/bubbles. Put collection device down.	Fill as accurate as possible	Wash your hands again with soap and water.

Open tube with blue liquid. Carefully pour in the saliva.





Fi n





ands again nd water.

**Disinfect tube, screw cap** and work surface. Ready for transport to the lab.



## Watch the instruction video

Scan QR code



Why wait any longer, just try and experience our solutions for saliva sampling!

Did you know that **90% of patients prefer saliva collection with Blue Collect™** instead of a nasopharyngeal swab for COVID-19 testing?

Product codes

Blue Collect™

REF IB\_CD01

Further reading
Performance details

P 14&15

# Performance details

InActiv Blue®

#### INACTIV BLUE®: VIRUS INACTIVATION PERFORMANCE

SARS-CoV-2 virus inactivation performance has extensively been tested by an independent laboratory (FARAH Research Center, Department of Veterinary Pathology, University of Liège, Belgium). The TCID50 method to measure virus inactivation is a modified assay based on chemical inactivation studies of SARS-CoV-1 (Darnell et al., 2004; Darnell and Taylor, 2006).

During validation it was demonstrated that the formulation of InActiv Blue<sup>®</sup> consistently results in a complete SARS-CoV-2 virus inactivation (log reduction:  $\geq$ 6). Long- time exposure of InActiv Blue<sup>®</sup> at 55 °C did not change the outcome of the test, which means that the formulation is extremely heat stable. SARS-CoV-2 virus is an enveloped virus, and non-enveloped viruses can be more resistant to high temperatures and disinfectant. Therefore, InActiv Blue<sup>®</sup>'s capability to inactivate a non-enveloped virus was also tested. The human norovirus is a small non-enveloped RNA virus. It is the most common cause of acute gastroenteritis (diarrhea and vomiting). This virus is notorious for its resistance to many inactivation methods. Because the human norovirus cannot be cultured, the murine norovirus was used for the inactivation experiments. An independent laboratory successfully achieved complete inactivation of murine norovirus using InActiv Blue<sup>®</sup> (>4.24 log reduction in TCID50 value).

In conclusion, InActiv Blue<sup>®</sup> collection tubes do not only completely inactivate SARS-CoV-2, but also other, even more inactivationresistant viruses. As a result, your personnel is better protected, all the way from collection to processing of the samples.

Each newly produced batch of InActiv Blue<sup>®</sup> is subject to the SARS-CoV-2 virus inactivation performance test. A batch is only released to the market if complete virus inactivation is shown after spiking with a virus stock solution that ensures at least a log reduction  $\geq 6$ .

#### INACTIV BLUE® AS A RELIABLE MEDIUM TO DETECT SARS-COV-2 RNA, INFLUENZA A/B & RSV A/B

Several clinical labs have demonstrated excellent comparative RT-qPCR test performance of InActiv Blue® against UTM. A summary of results is outlined in Figure 1-3.

Brief overview of test set-up: patient samples in standard transport medium of (nasopharyngeal) swabs that were previously tested positive for SARS-CoV-2, influenza A/B or RSV A/B, are diluted in InActiv Blue® or standard medium. Samples were subsequently stored at room temperature and retested after 1 day to determine the PCR quantification cycle (Cq) for SARS-CoV-2, influenza A/B or RSV A/B respectively. Figure 1-3 indicate a perfect concordance over a broad range of the Cq values.



#### **STABILITY OF SARS-COV-2 RNA ENSURED**

#### Storage of samples up to 30 days

Since (unexpected) delays in transport and testing can occur. it is important that a virus transport medium will ensure RNA stability and detectability for at least one week. As tested in two independent labs, InActiv Blue® medium was found to perfectly stabilize viral RNA for at least 8 days when samples are stored between 2-37 °C (Figure 4-6). Furthermore, sample RNA stability is ensured up to 30 days when stored between 2-25 °C as indicated in Figure 6.

Brief overview of test set-up: patient samples in standard transport medium of (nasopharyngeal) swabs that were previously tested positive for SARS- CoV-2, were diluted in InActiv Blue® or standard medium on day 0. Samples were subsequently stored at room temperature (RT), 4 °C or at 37 °C and retested on day 1, day 4, 8 and 30 to determine the PCR quantification cycle (Cq) for SARS-CoV-2. The figures below indicate the Co values obtained for a total of 119 samples tested in 2 different laboratories

Freeze-thaw stability of samples

InActiv Blue<sup>®</sup> medium perfectly supports your needs when samples must be stored for a longer time. Test data demonstrate that the stability of viral RNA is not affected by repeated freeze-thaw cycles.

Brief overview of test set-up: patient samples in standard transport medium of (nasopharyngeal) swabs that were previously tested positive for SARS-CoV-2, were diluted in InActiv Blue<sup>®</sup> on day 0. These samples were subsequently tested on day 0 and following 5 freeze-thaw cycles performed over 6 days. Figure 7 shows that the Cg values remain stable!

stored at 37 °C for 8 days (n= 12)

day 4

days post infection

day 8



stored at 4 °C and RT for 30 days (n=12)



#### Figure 4: Stability of SARS-CoV-2 RNA in InActiv Blue® stored at RT for 8 days, compared to storage in UTM (n=95)34

day 4

days post infection

32.

30

28

26

24 22-

20-

18

day 1

--- InActiv Blue-RT-high positives

---- InActiv Blue-RT-low positives

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28-

26

24

22-

20-

day 1

---- RT- high positives

--- RT - low positives

day 8

#### STABILITY OF INFLUENZA A/B - RSV A/B RNA

Additional data collected in collaboration with Sciensano confirm that InActiv Blue<sup>®</sup> is equally able to stabilize RNA of other common respiratory viruses: influenza A/B and RSV A/B.

Brief overview of test set-up : patient samples in standard transport medium of (nasopharyngeal) swabs that were previously tested positive for respectively influenza A/B or RSV A/B, were diluted in InActiv Blue<sup>®</sup> or standard medium on day 0. These samples were subsequently tested on day 1, 2, 3, 4, 7 and 8. Figure 8 illustrates that Cq values remain stable (8 days tested), Figure 9 shows that Cq values are similar (or even more sensitive) in InActiv Blue<sup>®</sup> medium compared to standard UTM medium.



#### Figure 10: 35-30 ------ទី 25-20 day 0 day 1 day 3 day 7 day 30 days post infection 50% v/v saliva - SARS-CoV-2 40% v/v saliva - SARS-CoV-2 30% v/v saliva - SARS-CoV-2 -4--50% v/v saliva - GADPH 40% v/v saliva - GADPH

#### ---- 30% v/v saliva - GADPH

#### USE OF SALIVA SAMPLES STORED IN INACTIV BLUE®

Tests on saliva can play an important role to have control over the COVID-19 pandemic. Massive use of preventive screening saliva tests can help to identify patients that spread the disease without having symptoms. In addition, saliva is a suitable alternative for a nasopharyngeal swab, especially for children (Jonckheere et al., Journal of Pediatrics, Perinatology and Child Health, 6:042-053, 2022). Data below indicates that SARS-Co-V-2 RNA remains stable for at least 30 days at room temperature in saliva samples stored in InActiv Blue<sup>®</sup>.

Brief overview of test set-up: 11 healthy donors were refrained from eating, drinking, smoking and using chewing gum for at least 30 min prior to saliva collection. In total, 54 ml of saliva was pooled and vortexed to prepare different dilutions of saliva in InActiv Blue® ranging from 30% to 50% saliva (v/v). On day 0, all tubes were spiked with viral-like particles to obtain a final low concentration of 100 000/ml. One set was stored at room temperature, the other set in the fridge at 4 °C. The PCR quantification cycle (Cq) for SARS-CoV-2 RNA was determined for all conditions on day 0, day 1, day 3, day 7 and day 30. Figure 10 shows that saliva is also a suitable specimen type for diagnostic testing on SARS-CoV-2: viral RNA of the E-gene and a human endogenous gene (GADPH) remains perfectly stable for at least 30 days. No difference is found between samples stored at RT or 4 °C (data not shown).

#### COMPATIBILITY WITH PLATFORMS

While labs are responsible for validating downstream RNA extraction and molecular testing, a non-exhaustive list of platforms that have successfully diagnosed patient swab samples stored in InActiv Blue® is available below:

instrument	RNA extraction	RT-qPCR		
Abbott Alinity M	Alinity m Sample Prep Kit 1 #09N18-001	Alinity m SARS-CoV-2 AMP kit #09N78-090		
CFX96 (Bio-Rad)	Real-Prep Viral DNA/RNA kit (BioSewoom)	STANDARD M nCoV Real-Time Detection kit (SD BIOSENSOR #11NCO10)		
CFX96 Deep Well Real Time PCR detection System (Bio-Rad) Maelstrom 9600 (Tanbead)	Tanbead Nucleic Acid Extraction Kit (96) #W665A10	Allplex TM 2019 nCov Assay RP4520D59		
CFX96 Deep Well Real Time PCR detection System (Bio-Rad) Microlab STARlet IVD (Seegene/Hamilton)	STARMag96x4 viral DNA/RNA 200c kit #EX00013C	Allplex TM 2019 nCov Assay RP4520D59		
CFX384 (Bio-Rad #1855485)	Zymo Research's Quick-RNA 96 #R1053	Bio-Rad iTaq one-step RT-qPCR mix #1725141		
	Norgen Biotek's Total RNA Purification 96-well Kit #24370	Bio-Rad iTaq one-step RT-qPCR mix #1725141		
	magtivio's MagSi-NA Pathogens #MDKT00210960	Bio-Rad iTaq one-step RT-qPCR mix #1725141		
Chemagic 360 Janus qPCR Janus reformatter (G3) Perkin Elmer QuantStudio7flex	Chemagic Viral DNA/RNA Kit special H96 #CMG-1033-S	Sars-CoV-2-RT-qPCR Reagent kit #3501-0010		
EMAG (Biomérieux) Stratagene MX3000-3005 (Agilent)	NUCLISENS easyMAG (Biomérieux #280130-35; #280146; #200292)	Superscript III Platinum One-Step qRT-PCR system (Invitrogen #11732-088)		
Hamilton Starlet	96X4 viral DNA/RNA 200 C kit #EX00013C	Allplex SARS-CoV-2 #RV10248X		
Hamilton Starlet/Tanbead	OptiPure Viral Auto Plate #W665A10	Allplex SARS-CoV-2 #RV10248X		
Lightcycler 480 (Roche)	MagnaPure 96 and Viral NA Small volume kit 0654388001	LightMix Modular Sarbecovirus SARS-CoV2 PCR kit #50-0776-96		
cobas 6800 system (Roche)	cobas omni reagent for sample preparation	cobas SARS-CoV-2: #09175431190 cobas Utility Channel with probes and primers for SARS -CoV-2 E gene		
Kingfisher FLEX (Thermofisher) Quantstudio 5 (Thermofisher)	Thermofisher's MagMAX Viral/Pathogen II kit #A48383	TaqPath COVID-19 CE-IVD RT-PCR kit #A48067		
Kingfisher FLEX (Thermofisher) Quantstudio 7 (Thermofisher)	Thermofisher's MagMAX Viral/Pathogen II kit #A48383	TaqPath COVID-19 CE-IVD RT-PCR kit #A48067		
cobas Liat PCR system (Roche)	COBAS SARS-CoV-2	COBAS SARS-CoV-2		
Lightcycler 96 (Roche)	magtivio's MagSi-NA Pathogens #MDKT00210960	Bio-Rad iTaq one-step RT-qPCR mix #1725141		
BioFire FilmArray (Biomérieux)	BioFire Respiratory 2	2.1 plus Panel (Biomérieux)		

### InActiv Blue<sup>®</sup> lowers viscosity of mucus-rich samples

As such, pipetting of mucus-rich biofluids like swab transport medium, saliva and sputum is improved when collected or diluted in InActiv Blue<sup>®</sup> buffer, because of its mucolytic properties.

#### VISCOSITY

Swab transport medium, saliva or sputum display increasing viscosity related pipetting challenges in the laboratory. To evaluate the mucolytic properties of InActiv Blue<sup>®</sup>, we have diluted surrogate sputum samples (20% (w/v) porcine mucin in water (Sigma #M1778)) either in water (50% v/v) or in InActiv Blue<sup>®</sup> (50% v/v). After 15 seconds vortexing, sputum samples were incubated for 1 hr at room temperature, followed by viscosity measurements using a Fluidicam<sup>RHEO</sup> (Formulaction-France) at 20 °C over a wide range of shear rates (250-3500 s<sup>-1</sup>).

Swab transport medium, saliva and sputum are non-Newtonian fluids (shear thinning fluids), meaning that their viscosity decrease as a function of the applied shear rate, which depends on the pipetting method (tip size and aspiration/ dispensing speed). At room temperature and over a relevant regime of shear rates, InActiv Blue® displays 14-19% lower viscosity compared to water.

shear rate (s <sup>.1</sup> )	water	InActiv Blue®
300	36.9 100%	31.7 86%
900	33.5 100%	28.0 84%
2700	30.1 100%	24.3 81%

viscosity of sputum diluted (50% v/v) (mPa.s)



# Saliva clinical study







# Equivalence of saliva RT-qPCR testing to nasal-throat/nasopharyngeal swab testing in the general practitioner's setting to detect SARS-CoV-2

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#### ABSTRACT

- *Study design:* Saliva has been proposed as valid alternative for nasopharyngeal swab for RT-qPCR detection of SARS-CoV-2. The sensitivity is generally equivalent, and it comes with much less discomfort for the patient. While there is an overall good performance in the literature for adults, there is much less information on the use of saliva in children or in the general practitioner's setting.
- *Methods:* We tested a novel commercially available saliva collection kit with a virus inactivating and RNA stabilizing buffer (InActiv Blue®) in matched saliva and swab samples from 245 individuals, including 216 children, collected by general practitioners.
- **Results:** Blind RT-qPCR testing of the saliva samples confirmed all 23 positives identified by swab testing (100% concordance), irrespective of age, presence of symptoms, or high-risk status. One child's saliva sample was found low positive while negative on the nasopharyngeal swab, resulting in an overall relative sensitivity of RT-qPCR saliva testing of 104.3%.
- Conclusion: Saliva collected in InActiv Blue<sup>®</sup> can be a valid alternative for SARS-CoV-2 RT-qPCR testing in the general practitioner's setting, including children.

To control the COVID-19 pandemic, one needs to stop the spread of the SARS-CoV-2 virus by identifying and isolating infectious individuals. While a PCR test on a nasopharyngeal swab is generally considered to be the most sensitive diagnostic test, it comes with a few important shortcomings, such as discomfort for the patient (in particular, but not limited to children), the necessity for a trained healthcare professional to take a sample, risk for nosocomial virus transmission, and the identification of SARS-CoV-2 positive patients that are no longer infectious<sup>1</sup>. As COVID-19 is an airborne disease due to virus-laden aerosols expelled by an infectious individual<sup>2</sup>, several studies have evaluated saliva as an alternative and more easily accessible sample to detect SARS-CoV-2. In a meta-analysis, PCR testing on saliva yielded a sensitivity and specificity comparable to nasopharyngeal swab testing in ambulatory patients presenting with minimal or mild symptoms<sup>3</sup>.

Given the ease of sample collection and increased patient comfort, the authors suggest that laboratories should consider adopting saliva as their first sample choice, especially in screening programs. In a more recent systematic review and meta-analysis, saliva PCR testing was specifically evaluated in children<sup>4</sup>. Comparable performance of saliva to nasopharyngeal samples was shown in both symptomatic and asymptomatic pediatric patients. While in general the RT-qPCR SARS-CoV-2 detection sensitivity and specificity on saliva is good, the various studies are quite heterogeneous in terms of patient inclusion criteria, volume of saliva collected, and saliva collection and preservation method. In our study, we aimed to evaluate a new saliva collection kit for self-sampling of a small volume of saliva under supervision using a virus inactivating and RNA stabilizing medium at the general practitioner's office, with a focus on children. In total, matched swab and saliva was taken from 245 individuals, including 216 children.

#### **STUDY SETUP AND RESULTS**

In a first patient cohort, matched saliva and swab samples were collected by the general practitioner (GP) during visit of 209 children aged 5-16 years (median age of 9 years), because of high-risk contact and/or COVID-19 symptoms (May-June, 2021). While participants of the study were asked not to eat/drink/smoke/use chewing gum/candy/ mint 30 min prior to saliva collection, and to rinse the mouth with water 10 minutes prior to saliva collection, and to rinse the mouth with water 10 minutes followed these recommendations. Also, while children were asked to produce deep throat saliva (posterior oropharyngeal saliva) by scraping the throat, this was not an inclusion criterion if the participant could not produce such a sample. Upon collection, patient material was virus-inactivated and RNA-stabilized by InActiv Blue® medium and picked up by medical lab 1 for routine RT-qPCR testing of SARS-CoV-2 on the nasopharyngeal or combined nasal/throat swab sample. Eleven swab samples (5.26%) were tested positive; these 11 positive and 62 (of the 198) randomly selected negative patients were sent for blind analysis to lab 3 using a validated RT-qPCR testing procedure for saliva (Figure 1).

A second cohort of 36 symptomatic individuals (including 7 children) was selected by other GPs for matched sampling of saliva and nasopharyngeal swab, using the same instructions as described for the first cohort (May-June, 2021). Upon collection, patient material was virus-inactivated and RNA-stabilized by InActiv Blue® medium and picked up by medical lab 2 for routine RT-qPCR testing of SARS-CoV-2 on the swab sample. Twelve swab samples (33%) were tested positive; all 36 saliva samples were sent for blind RT-qPCR analysis to lab 3 (Figure 1).

#### Figure 1: STARD diagram displaying study setup of matched saliva and nasopharyngeal or nasal/throat swab sampling of 245 patients at the general practitioner's setting, including 216 children (209 from lab 1 and 7 from lab 2).



 Table 1: Demographic information of patients included in SARS-CoV-2 RT-qPCR testing on saliva

 # patients
 # swab positive

 # patients
 # swab positive

200	5-16 years	80	12	13
age	≥17 years	29	11	11
symptoms	yes	92	19	20
symptoms	no	17	4	4
high-risk	yes	35	16	17
contact(s)	no	74	7	7

All 23 swab positive samples tested positive using saliva (Table 2, Table 7); in other words, the proportion of saliva-positive samples among swab-positive samples is 100% ([83.1-100.0%] 95% confidence interval), including all swab-positive children's samples (n=12, [71.8-100.0%] 95% confidence interval, Table 3), all adult samples (n=11, [70.0-100.0%] 95% confidence interval, Table 4), all symptomatic samples (n=19, [80.2-100%] 95% confidence interval, Table 5), and all asymptomatic samples (n=4, [45.4-100%] 95% confidence interval, Table 6).

Lab 3 applied a validated RT-qPCR test procedure on saliva from all 23 positive and a randomly selected set of 86 negatives cases from the 2 cohorts. The demographic information of all 109 saliva samples are mentioned in Table 1.





Saliva from the 86 swab-negative patients was confirmed to be negative for all but one sample. One saliva sample from a 10-year-old child was tested low positive (Cq=31.1, Table 7, patient ID F), 7 days after developing COVID-19 symptoms. Hence, the relative sensitivity of RT-qPCR saliva testing was 104.3% across all patients.

While direct quantitative comparison of Cq values across laboratories is not recommended, the Cq values between swab and saliva are largely comparable, with a median difference of 1.9 cycles in favor of the swab result (Figure 2).

To assess the impact of eating/drinking or rinsing the mouth prior to saliva collection on the SARS-CoV-2 RT-qPCR detection sensitivity, we compared the difference in Cq value of the spike-in RNA control (as a measure for inhibition) between these 2 groups, or the difference between the saliva Cq and the swab Cq value (delta-Cq) for SARS-CoV-2 between these groups. Both analyses provide no evidence that eating or drinking 30 minutes prior to saliva collection negatively affect SARS-CoV-2 detection sensitivity (p-values > 0.05). With the smallest group size being 18 and an observed standard deviation of spike-in RNA Cq of 0.375, we had >95% power to detect a 0.5 cycle difference.

#### Table 7: List of patients with positive saliva RT-qPCR result (N/A, not available)

lab ID	patient ID	saliva Cq	symptoms	high-risk	age	days symptoms	ate/ drank	rinsing mouth	swab Cq (gene)	swab concentration copies/ml	swab type
1	Α	19.0	yes	yes	11-16	N/A	no	N/A	22.4 (E)	10 <sup>5</sup> -10 <sup>7</sup>	nasopharyngeal
1	В	19.9	yes	yes	5-10	1	no	no	24.1 (E)	10 <sup>5</sup> -10 <sup>7</sup>	nasopharyngeal
1	С	22.8	yes	yes	5-10	2	no	no	26.5 (E)	10 <sup>3</sup> -10 <sup>5</sup>	nasal/throat
1	D	23.8	yes	yes	5-10	N/A	no	N/A	25.5 (E)	10 <sup>3</sup> -10 <sup>5</sup>	nasopharyngeal
1	E	27.3	yes	yes	5-10	1	no	no	18.3 (E)	≥ 10 <sup>7</sup>	nasal/throat
1	F	31.1	yes	yes	5-10	7	yes	no	N/A	N/A	nasopharyngeal
1	G	33.3	yes	yes	5-10	N/A	no	no	33.9 (E)	<103	nasopharyngeal
1	Н	19.1	no	yes	5-10	N/A	no	no	27.1 (E)	10 <sup>3</sup> -10 <sup>5</sup>	nasopharyngeal
1	I	21.6	no	yes	11-16	N/A	yes	no	23.1 (E)	10 <sup>3</sup> -10 <sup>5</sup>	nasopharyngeal
1	J	23.6	no	yes	5-10	N/A	yes	no	25.4 (E)	10 <sup>5</sup> -10 <sup>7</sup>	nasal/throat
1	К	25.6	no	yes	5-10	N/A	no	no	23.0 (E)	10 <sup>5</sup> -10 <sup>7</sup>	nasopharyngeal
1	L	23.3	yes	no	5-10	1	no	no	26.2 (E)	10 <sup>3</sup> -10 <sup>5</sup>	nasal/throat
2	М	13.9	yes	yes	40-49	2	no	yes	11.2 (N)	N/A	nasopharyngeal
2	Ν	19.0	yes	no	17-29	2	no	yes	17.6 (E)	N/A	nasopharyngeal
2	0	19.7	yes	no	60-69	4	no	no	23.0 (E)	N/A	nasopharyngeal
2	Р	20.2	yes	yes	17-29	2	no	no	9.3 (N)	N/A	nasopharyngeal
2	Q	20.8	yes	no	30-39	1	no	no	13.6 (N)	N/A	nasopharyngeal
2	R	24.1	yes	yes	30-39	1	yes	no	12.2 (N)	N/A	nasopharyngeal
2	S	24.4	yes	no	17-29	1	no	no	15.9 (N)	N/A	nasopharyngeal
2	T	24.8	yes	yes	30-39	1	no	yes	31.4 (E)	N/A	nasopharyngeal
2	U	25.5	yes	no	17-29	3	no	yes	29.5 (E)	N/A	nasopharyngeal
2	V	28.4	yes	yes	11-16	2	no	no	18.7 (N)	N/A	nasopharyngeal
2	W	32.2	yes	yes	30-39	2	no	no	24.9 (E)	N/A	nasopharyngeal
2	Х	33.8	yes	no	50-59	N/A	no	yes	31.9 (E)	N/A	nasopharyngeal

#### DISCUSSION

PCR-based testing for SARS-CoV-2 has been instrumental in the global effort to control the COVID-19 pandemic. While nasopharyngeal swabs are widely recommended to maximize detection sensitivity, this sampling procedure comes with significant discomfort, especially for children, and requires trained staff for collection. Furthermore, maximizing diagnostic sensitivity may not be the best strategy to prevent spreading; instead, frequency of testing should be prioritized over sensitivity in controlling the spread of this virus<sup>5</sup>. Saliva may provide an excellent alternative for a swab as it allows non-invasive and repeated self-collection and has been demonstrated to result in equivalent sensitivity<sup>3,4</sup>. Nonetheless, noticeable performance differences among individual studies are published, likely resulting from varying collection devices, with or without stabilizing medium (presumably important because of large amounts of RNases in saliva), sample storage conditions, time delays between collection and testing, phase of the pandemic<sup>6</sup> during which sampled are collected, donor inclusion criteria (hospitalized vs. asymptomatic persons), and unstandardized laboratory saliva testing. Also, different ways of saliva collection are reported, including spitting (either or not stimulated), gargling, or posterior oropharyngeal spitting (throat clearing), and varying recommendations to refrain from eating or drinking, and rinsing the mouth prior to collection.

In our study, we used a novel saliva collection device for supervised self-collection of a small volume of 1.3 ml unstimulated saliva in a stabilizing buffer that inactivates infectious agents and stabilizes RNA. A small volume of 1.3 ml saliva is an important benefit, especially for children and elderly people, who have great difficulties in producing large saliva volumes. Most published studies require at least 2 and up to 5 ml of saliva. In our study, we included children from the age of 5 years onwards as they can easily produce a 1.3 ml spitting sample, an age cut-off also recommended by Delaney et al.7.

While our patient cohort size is modest, our results are perfectly in line with recent meta-analyses on the use of saliva as an alternative to nasopharyngeal swabs<sup>3,4</sup>. We observed a 100% concordance, across all demographic groups, irrespective of age, presence of symptoms, or high-risk status. Our study has not observed any false negatives, and -despite the difficulties to compare Cq values across laboratories- general good concordance in Cq values between saliva and swab. In line with previous reports (reviewed in 3), we have detected one case that is saliva positive and swab negative. Of note, this child was sampled 7 days after symptoms started, the longest period in our cohort. It remains to be determined whether the higher relative sensitivity observed for saliva is due to variation in nasopharyngeal sampling<sup>10</sup> or due to differential viral load dynamics over time in function of body part<sup>10</sup>.

While neat saliva may pose handling challenges because of its complex matrix with non-Newtonian behavior and high viscosity, we did not observe any pipetting problems in our study. One possible explanation may be the reported mucolytic effect of some components of the InActiv Blue® transport medium, such as guanidine thiocyanate and sarkosyl<sup>8,9</sup>. We did also not observe any signs of RT-qPCR inhibition or loss of sensitivity when comparing patients with respect to their eating/drinking or mouth rinsing behavior prior to saliva collection. Together with the 100% concordance rate, our results therefore suggest that the reported recommendations to refrain from drinking or eating 30 minutes prior to saliva collection or rising the mouth with water 10 min prior to collection may not be universally valid.

While not specifically tested in this study, saliva also holds promise to detect other respiratory viruses, like RSV and influenza<sup>12</sup>. This may be of great value for differential diagnosis of SARS-CoV-2, RSV and influenza using the same saliva sample.

#### MATERIALS AND METHODS

The study was approved by the Ghent University Hospital ethics committee (B6702021000459) for parallel collection of saliva from children aged 5-16 and adults during a visit at the general practitioner (GP) during which a swab is collected for diagnostic purposes. At the same time, for each patient, a short survey is completed to enquire about symptoms, high-risk contacts, and eating/drinking behavior or mouth rinsing with water prior to saliva collection.

At the GP, saliva was collected using the commercially available CE marked Saliva Collection Kit (InActiv Blue<sup>®</sup>, IB\_COL) according to the kit's instructions ( $\sim$ 1.3 ml saliva + 2 ml InActiv Blue<sup>®</sup>) and a nasopharyngeal (lab 1, lab 2) or combined nasal/throat swab (lab 1) was collected in 2 ml VST medium (#456162, Greiner Bio-One; lab 1) or in 2 ml of InActiv Blue<sup>®</sup> (#456604, InActiv Blue<sup>®</sup>; lab2). InActiv Blue<sup>®</sup> is a virus inactivating and RNA stabilizing buffer that protects RNA for up to 30 days at room temperature. Sample transport from the GP to medical lab 1 or 2 was performed at room temperature, followed by immediate processing of the swab sample according to the routine diagnostic procedure. The saliva samples were stored at 2-8 °C (lab 1) or frozen (lab 2) and shipped to lab 3 for further testing.

Upon arrival at lab 3, the samples were processed according to an ISO 17025 accredited procedure. The saliva samples were first thawed, and the tubes were put in an oven (Binder FP115) at 83 °C for a period of 10 to 20 minutes, depending on number of tubes, such that the cap reaches 70 °C for at least 5 minutes (heat camera verified). The heat procedure does not only guarantee complete inactivation of the thread of the screw cap (not exposed to the inactivating buffer) but may also help render the sample less viscous. Hundred µl of saliva was aspirated using a Tecan Freedom EVO 200 liquid handler, followed by MagSI-NA Pathogens RNA extraction (magtivio #MDKT00210960) on a PurePrep 96 instrument (magtivio) and eluted in 75 µl. Six µl of RNA eluate was used as input for a 20 µl duplex RT-qPCR reaction in a CFX384 qPCR instrument (Bio-Rad) using 10 µl One Step PrimeScript III (Takara Bio #RR600B) 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<sup>13</sup> and CDC N2 gene primers/probe<sup>14</sup> were used (both in 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).

In medical lab 1 (Labo Nuytinck), nasopharyngeal or nasal/throat samples were analyzed using a validated routine RT-qPCR test (Allplex SARS-CoV-2 Assay; Seegene, Accuramed), consisting of RNA extraction with STARMag 96X4 viral DNA/RNA 200C kit (Seegene, Accuramed) on a Hamilton Starlet followed by RT-qPCR on an CFX96 qPCR instrument (Bio-Rad). Seegene viewer v3 was used for amplification curve interpretation. For positive samples, the Cq values of E, RdRP/S and N genes were reported. Semi-quantitative swab viral concentration was calculated using reference material provided by the Belgian Reference Center.

In medical lab 2 (Medisch Labo Bruyland), nasopharyngeal samples were analyzed using a validated routine RT-qPCR test. Most of the samples were analyzed (n = 21) using the ThermoFisher platform (reporting N, S and ORF genes). Prior to the analysis, the nasopharyngeal samples were heat-inactivated and were transferred into (600 µl) transparent tubes. RNA was extracted from 200 µl sample using MagMAX Viral/Pathogen II Nucleic Acid Isolation kit (ThermoFisher), Tecan Freedom EVO 100 liquid handler (Tecan) and KingFisher Flex (ThermoFisher). The barcoded PCR plate was prepared using the RNA extracts, TaqPath COVID-19 CE-IVD RT-PCR kit (ThermoFisher) and Tecan Freedom EVO 100 liquid handler. PCR was carried out on a QuantStudio5 (ThermoFisher). Data were analyzed using the FastFinder software v4.5.2 (UgenTec). For the SARS-CoV-2 positive samples, the Cq values of N, S and ORF genes were reported. A minority of the samples (n = 15) was analyzed using the Roche Platform (reporting E gene). Prior to the analysis, the samples were transferred to flow tubes (appropriate for the Roche platform) and an equal volume of Cobas PCR Medium was added to inactivate the samples. RNA was extracted using the Flow primary sample handling pipetting robot (Roche/Hamilton), MagNA Pure 96 DNA and Viral NA Small Volume kit (Roche) and MagNA Pure 96 (Roche). The barcoded PCR plate was prepared using the RNA extracts, RNA process Control kit (Roche), LightMix Modular Sarbecovirus SARS-CoV-2 (Roche/TIB Molbiol) and Flow PCR Setup pipetting robot (Roche/Hamilton). PCR was performed using the ELightCycler 480 II. Data were analyzed using the FLOW software (Roche) and for the positive samples, the E gene Cq values were reported.

Cq values used in figures or mentioned in tables are E gene for lab 1, E or N gene for lab 2, and combined E/N gene for lab 3.

The relative sensitivity was calculated by dividing the sensitivity of testing on saliva by the sensitivity on swabs. The relative sensitivity can take values from zero to infinity. A value above one indicates that testing on saliva is more sensitive than on the swab. Confidence intervals on proportions of counts were calculated using GraphPad's QuickCalcs according to the modified Wald method. An unpaired t-test (using MS Excel version 16.52) was used to compare Cq values of the spike-in RNA between saliva samples from patients who either did or did not eat/drink 30 minutes prior to saliva collection, or who either did or did not rinse their mouth with water 10 minutes prior to saliva collection. The same test was used to compare delta-Cq values (saliva Cq – swab Cq for SARS-CoV-2 positive cases) between the aforementioned groups. Power analysis for a t-test was calculated using Piface version 1.76.

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