



Department of Health, Behavior and Society
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Oral fluid alternative for COVID-19

5.1.2e

webinar LCDK | 11-09-2020



Introduction

Change in policy

- Schools open again
- Accessible testing through municipal health service (GGD) testing streets
- Hesitance to test young children (< 6 yrs of age) using NP and OP swab
 - Burdensome for child
 - Reluctance parents
 - Reluctance specimen collector
- Oral fluid an option?

Saliva is more sensitive for SARS-CoV-2 detection in COVID-19 patients than nasopharyngeal swabs

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COVID-19 SARS-CoV-2
medRxiv and bioRxiv

Subject Area

Infectious Diseases (except

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Saliva is less sensitive than nasopharyngeal swabs for COVID-19 detection in the community setting

[David Becker](#), [Efren Sandoval](#), [Aakash Amin](#), [Peter De Hoff](#), [Amberly Diets](#), [Nicole Leonetti](#), [Yan Wei Lim](#), [Christie Elliott](#), [Louise Laurent](#), [Joseph Grzymiski](#), [James Lu](#)

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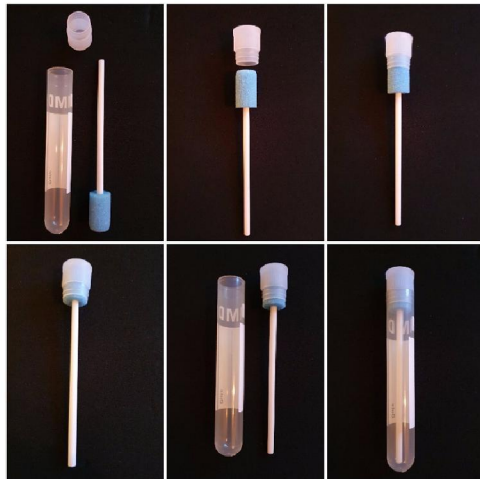


Design FFX household study

Subject	Day															Week
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15-21	
Start questionnaire	x															
Symptoms diary		x	x	x	x	x	x	x	x	x	x	x	x	x		
Final questionnaire																x
Blood (serum / cells)	x														x	x
Nose and throat swab	x														x	
Oral fluid	x														x	x
Feces	x														x	x
Optional nose and throat swab			x			x			x			x				



Specimen collection



Oracol S10

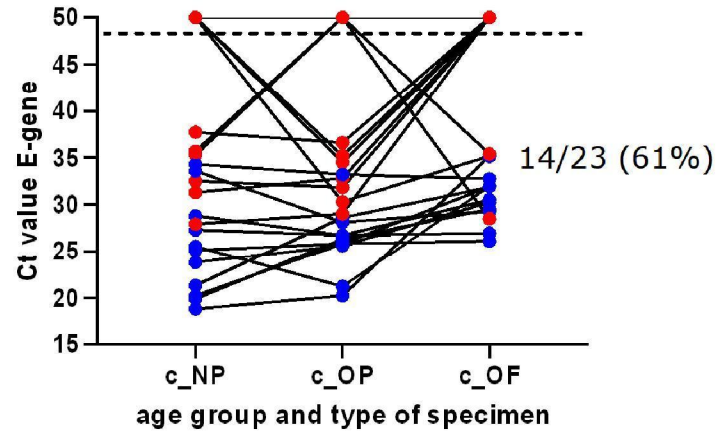
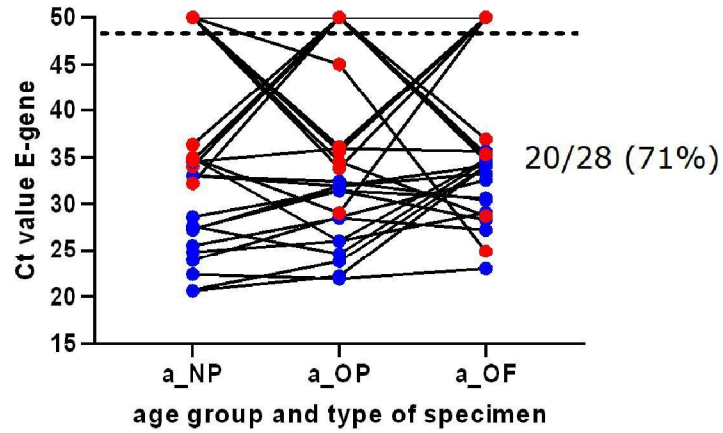
Protocol

- No brushing teeth, eating, drinking other than water and no smoking at least half an hour before specimen collection
- Insert Oracol sponge between cheek and teeth and keep it there about one minute moving back and forth
- Repeat with second sponge
- Transport cooled on wet ice to lab
- In BSC remove and invert sponge in tube
- Centrifuge 10 minutes 3,000 rpm
- Collect oral fluid and store at -80°C

- Thaw aliquot and keep cool
- Extract total NA using MagNA Pure MP96
- Perform RT-PCR for E-gene SARS-CoV-2 (Corman et al.)



Results oral fluid d1



a=adult (68); c=child (76); NP=nasopharyngeal; OP=oropharyngeal; OF=oral fluid



D1 NP and/or OP positive and OF alternative specimen

Adults				Children			
68 pairs				76 pairs			
Oral fluid				Oral fluid			
		+	-			+	-
Respiratory specimens	+	16	8	Respiratory specimens	+	12	9
	-	4	40	Respiratory specimens	-	2	53



D1 NP and/or OP positive and OF alternative specimen

NP/OP Ct ≥ 29
 7/8 sero pos; 1 seroconversion
 3/8 feces pos
 1/8 no further evidence

NP/OP Ct ≥ 27.9
 9/9 sero pos; 2 seroconversion
 6/9 feces pos
 1/9 no further evidence

Adults				Children			
68 pairs				76 pairs			
Oral fluid				Oral fluid			
		+	-			+	-
Respiratory specimens	+	16	8	Respiratory specimens	+	12	9
	-	4	40		-	2	53

OF Ct ≥ 34.6
 3/4 sero pos
 1/4 feces pos
 1/4 no further evidence

8/40 sero pos
 2/40 seroconversion

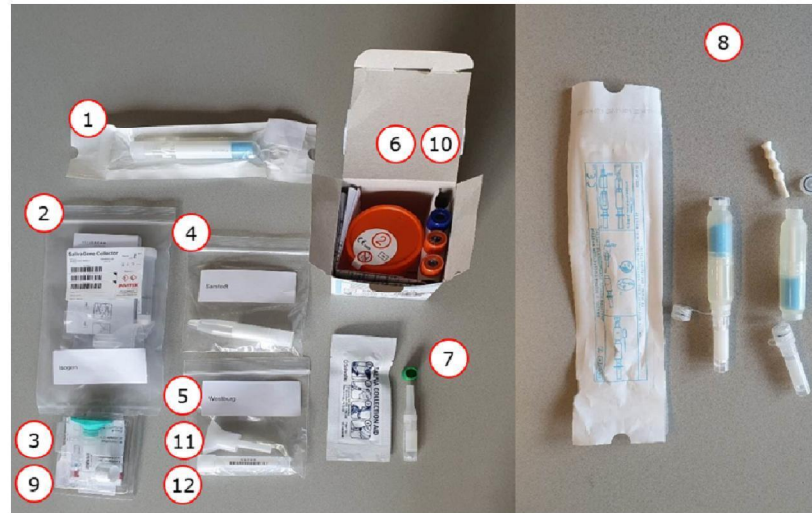
OF Ct ≥ 28.5
 2/2 no further evidence

6/53 sero pos
 4/53 seroconversion



Alternative saliva collection systems

System	Manufacturer	Model	Buffer
1	Malmed, UK	Oracol S10	No
2	INVITEK Molecular, Germany	SalivaGene Collector	Dried
3	NORGEN BIOTEK Corp, Canada	Saliva RNA Collection and Preservation Device	Not used
4	Sarstedt, Germany	Salivette® REF 51.1534	No
5	Isohelix, UK	Custom order	No
6	Greiner	Greiner Bio-One Saliva Collection System	Not used
7	Salimetrix, State College, USA	Saliva Collection Ad	No
8	Malmed, UK	Oracol S14	No
9	NORGEN BIOTEK Corp, Canada	Saliva RNA Collection and Preservation Device	Separate
10	Greiner	Greiner Bio-One Saliva Collection System	Separate
11	Isohelix, UK	GeneFiX DNA saliva collection	Yes
12	Isohelix, UK	GeneFiX RNA saliva collection	Yes



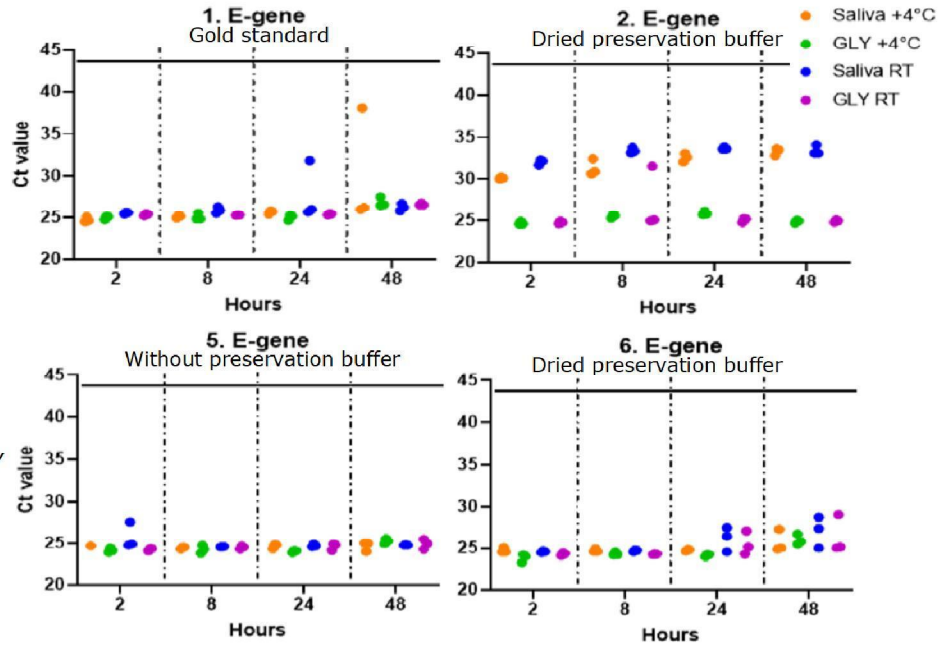
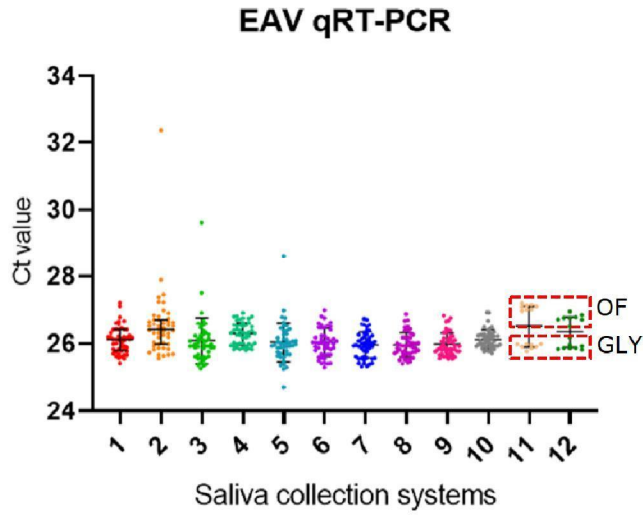


Elements relevant for selection

- Practical for children <6 yrs of age
- Stimulant for salivation
- DNA a/o RNA preservative
- Practical for sample collection team
- Safety for sample collection team
- Practical in laboratory (extra pipetting, centrifugation needed)
- Safety in laboratory
- Downstream analysis molecular as well as antibodies
- Spill hazard



Inhibition of amplification



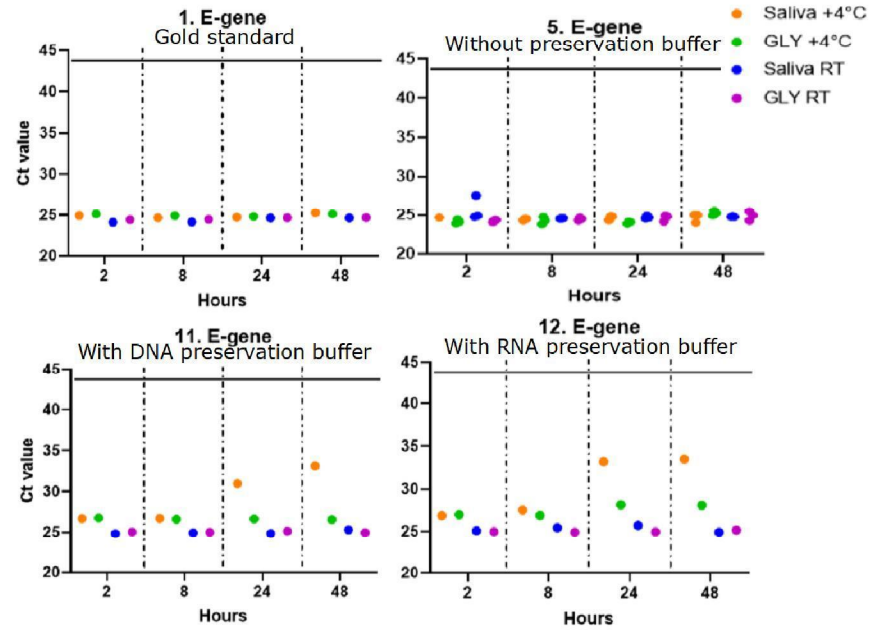


Variants Isohelix system



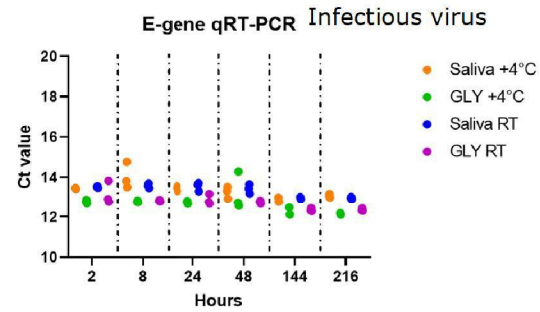
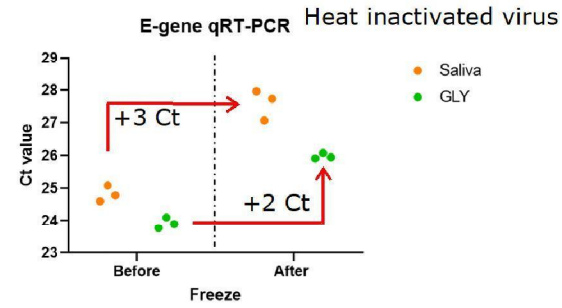
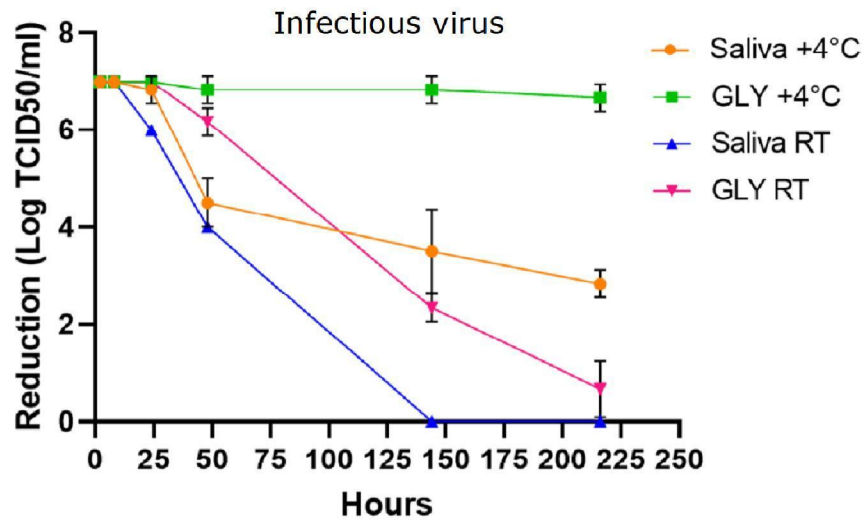
Without buffer
 With DNA preservation buffer
 With RNA preservation buffer

Manufacturer recommendation:
 With buffer don't store at 4°C



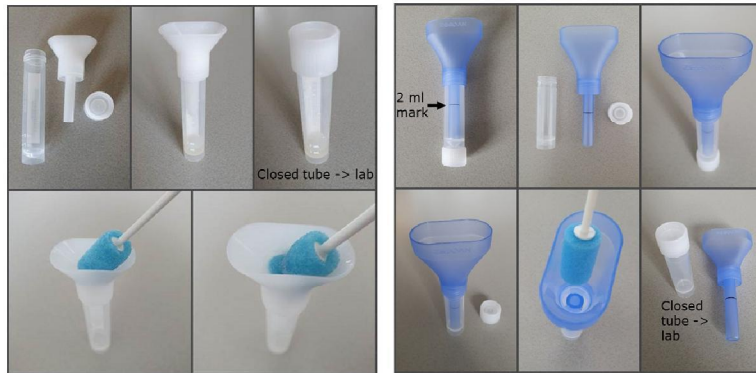


Storage oral fluid with intact SARS-CoV-2





How to use during collection and processing in the lab



Left: Funnel with tube: Isohelix, UK, custom order

Right: Funnel with tube: Zeesan, Xiamen, China, custom order
Oracol S10 sponge: Malmed, UK

Modified protocol:

- No brushing teeth, eating, drinking other than water and no smoking at least half an hour before specimen collection
- Insert Oracol sponge between cheek and teeth and keep it there about one minute moving back and forth
- Squeeze out the saliva in the funnel of the collection tube
- Repeat with (second) sponge until 2 ml is collected
- Transport at ambient temperature to the lab
- Asap in BSC aliquot and start extraction (short storage period at 4°C possible; don't freeze/thaw!)
- Perform RT-PCR for SARS-CoV-2



Conclusions

- Oral fluid good alternative to nasopharyngeal and oropharyngeal swabs
- (Very) low viral loads in NP and OP might be missed
- Feces is another not invasive alternative specimen; together with oral fluid high sensitivity
- In municipal health services testing streets oral fluid is most practical

- By combining sponge with collection tube with funnel collection processing in the lab of oral fluid specimen can conveniently be done
- DNA or RNA preservation buffers for OF do not have added value for SARS-CoV-2 detection
- Working with OF requires strict procedure in the labs to prevent loss of sensitivity; keep OF specimens cool, work quickly and do not freeze/thaw



Acknowledgements

- RIVM-IDS Virology

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- RIVM-EPI

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- RIVM-IIV

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