Oral fluid alternative for COVID-1

5.1.2e

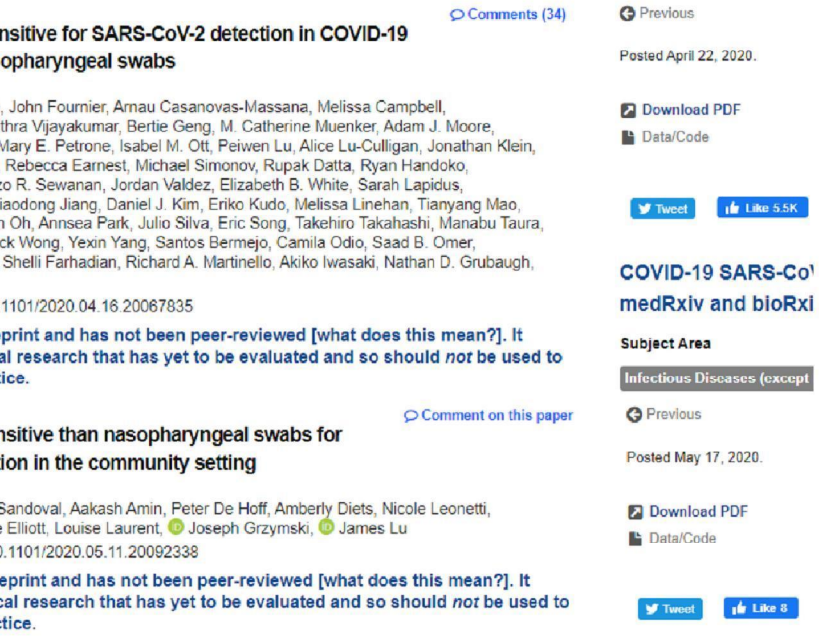
webinar LCDK | 11-09-2020

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?



Comments (34)

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

Anne Louise Wyllie, John Fournier, Arnau Casanovas-Massana, Melissa Campbell, Maria Tokuyama, Pavithra Vijayakumar, Bertie Geng, M. Catherine Muenker, Adam J. Moore, Chantal B. F. Vogels, Mary E. Petrone, Isabel M. Ott, Peiwen Lu, Alice Lu-Culligan, Jonathan Klein, Arvind Venkataraman, Rebecca Earnest, Michael Simonov, Rupak Datta, Ryan Handoko, Nida Naushad, Lorenzo R. Sewanan, Jordan Valdez, Elizabeth B. White, Sarah Lapidus, Chaney C. Kalinich, Xiaodong Jiang, Daniel J. Kim, Eriko Kudo, Melissa Linehan, Tianyang Mao, Miyu Moriyama, Ji Eun Oh, Annsea Park, Julio Silva, Eric Song, Takehiro Takahashi, Manabu Taura, Orr-El Weizman, Patrick Wong, Yexin Yang, Santos Bermejo, Camila Odio, Saad B. Omer, Charles S. Dela Cruz, Shelli Farhadian, Richard A. Martinello, Akiko Iwasaki, Nathan D. Grubaugh, Albert I. Ko

doi: https://doi.org/10.1101/2020.04.16.20067835

This article is a preprint and has not been peer-reviewed [what does this mean?]. It reports new medical research that has yet to be evaluated and so should *not* be used to guide clinical practice.

Comment on this paper

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 Grzymski, James Lu

doi: https://doi.org/10.1101/2020.05.11.20092338

This article is a preprint and has not been peer-reviewed [what does this mean?]. It reports new medical research that has yet to be evaluated and so should *not* be used to guide clinical practice.

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COVID-19 SARS-Co' medRxiv and bioRxi

Subject Area

Infectious Diseases (except

Previous

Posted May 17, 2020.

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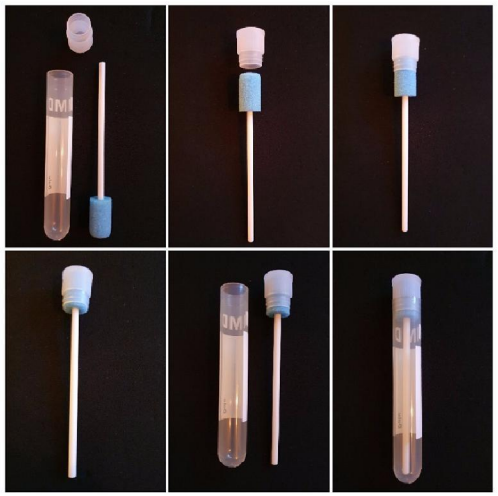
Design FFX household study

Subject	Day															Week
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15-21	4-6
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



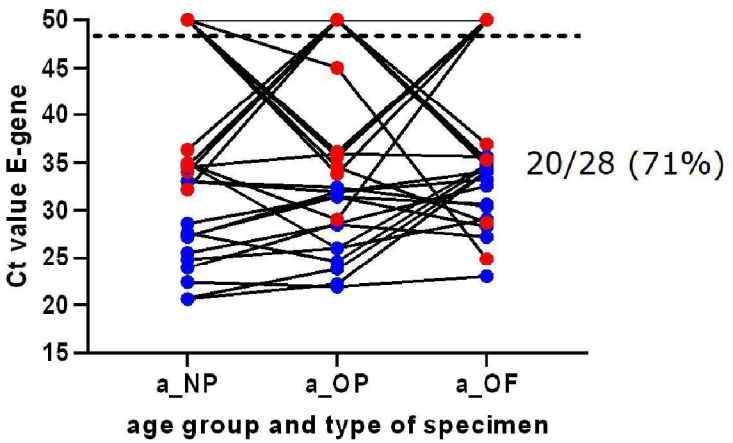
Oracol S10

Protocol

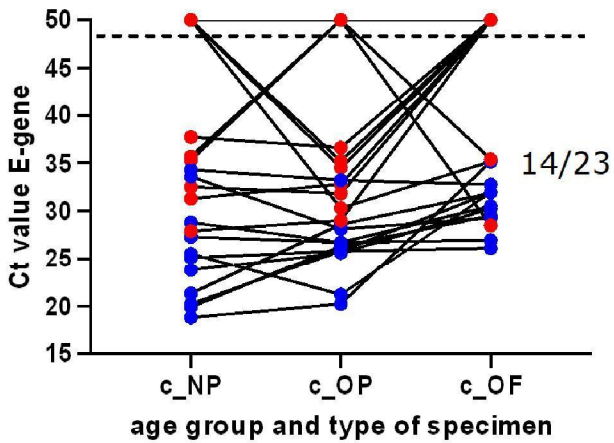
- No brushing teeth, eating, drinking other than water and no smoking at least half an hour before specimen collection
- Insert Oracol sponge between cheek and teeth and keep it there about one minute moving back and forth
- Repeat with second sponge
- Transport cooled on wet ice to lab
- In BSC remove 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



20/28 (71%)



14/23 (61%)

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		-	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

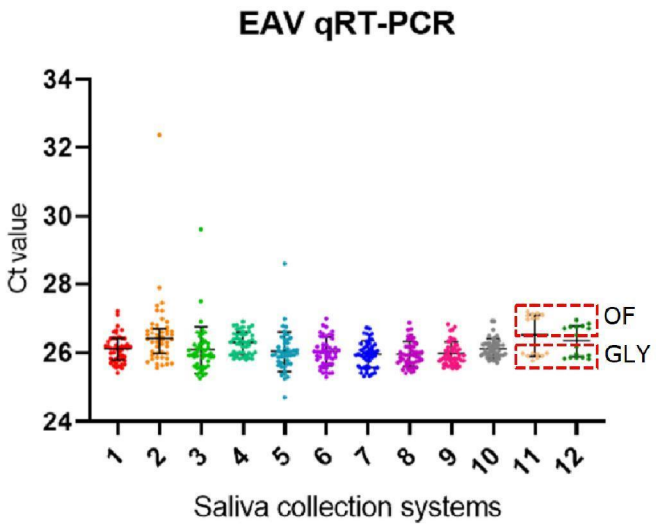
Alternative saliva collection systems

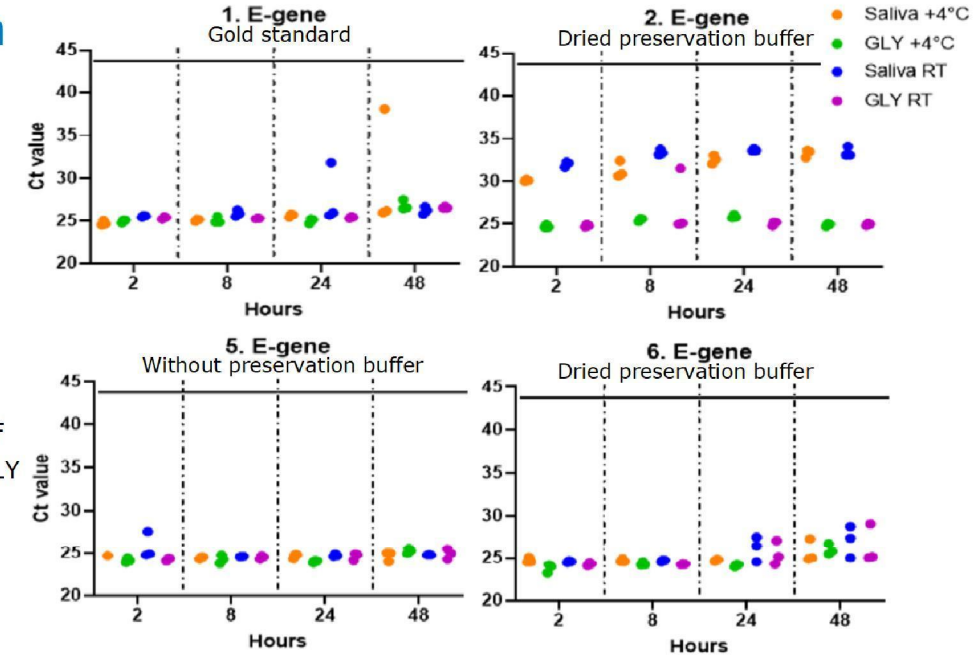
System	Manufacturer	Model	Buffer
1	Malmed, UK	Oracol S10	No
2	INVITEK Molecular, Germany	SalivaGene Collector	Dried
3	NORGEN BIOTEK Corp, Canada	Saliva RNA Collection and Preservation Device	Not used
4	Sarstedt, Germany	Salivette® REF 51.1534	No
5	Isohelix, UK	Custom order	No
6	Greiner	Greiner Bio-One 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

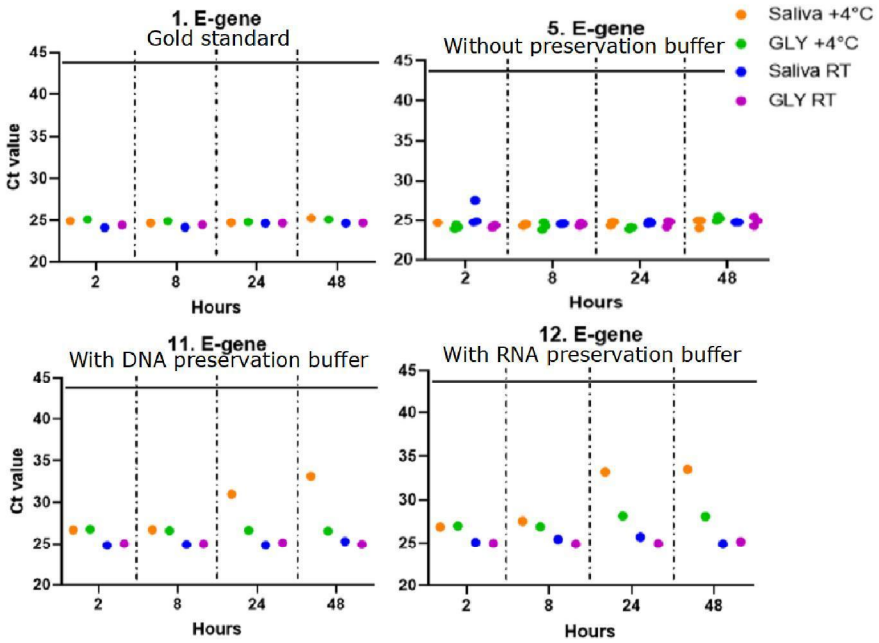




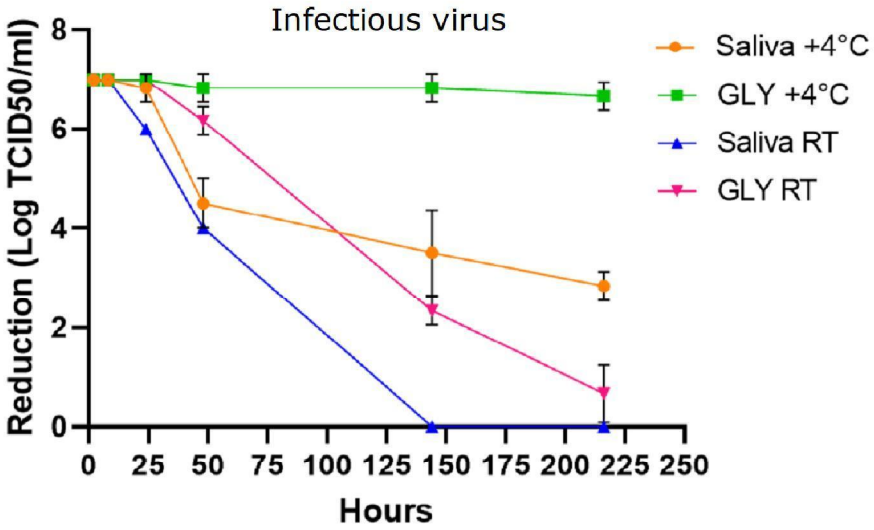
Variants Isohelix system

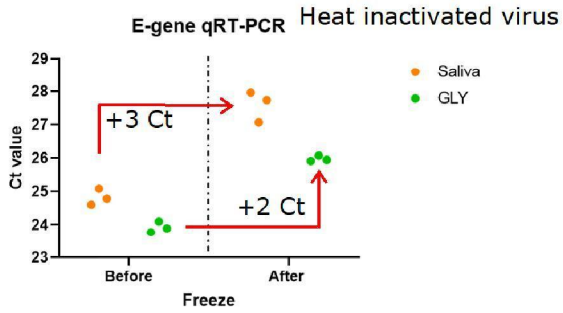
Without buffer
With DNA preservation buffer
With RNA preservation buffer

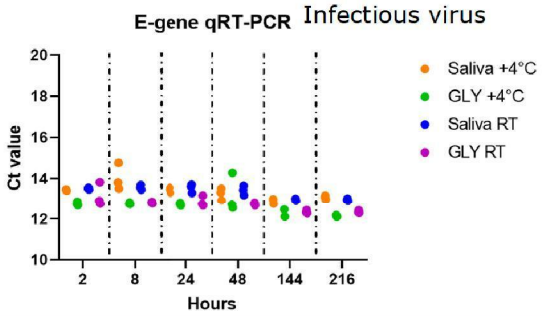
Manufacturer recommendation:
With buffer don't store at 4°C



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 alterative 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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