National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

Oral fluid alternative for COVID-19 diagnostics

(10)(2e)

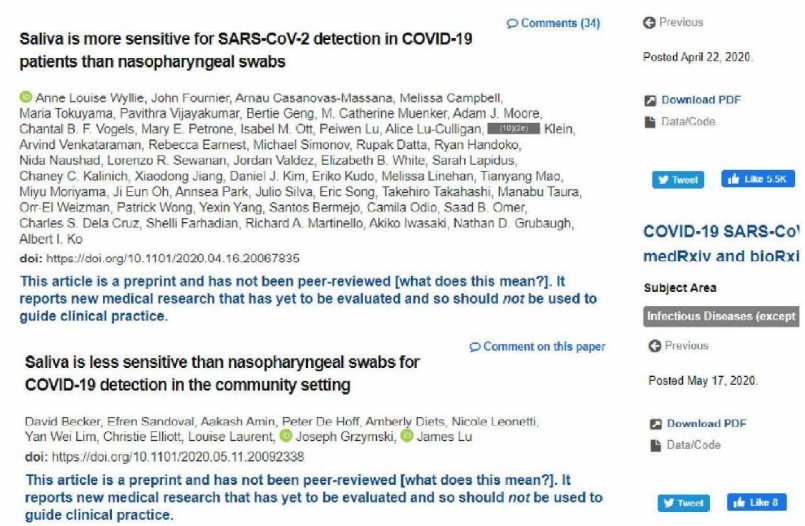
webinar WHO | 09-06-2020

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, [redacted] 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

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This article is a preprint and has not been peer-reviewed [what does this mean?].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This article is a preprint and has not been peer-reviewed [what does this mean?].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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Subject Area

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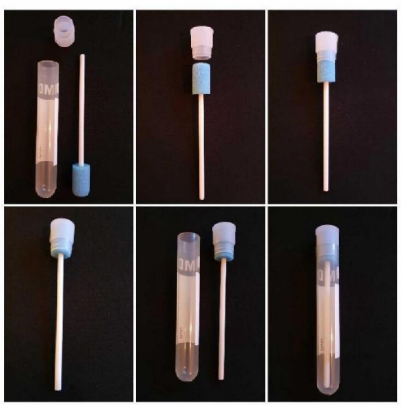
Design FFX household study

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



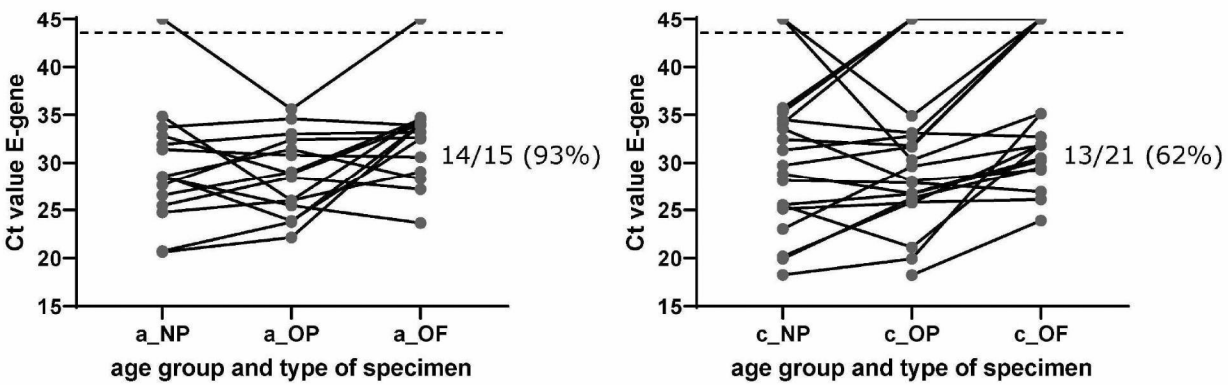
Oracol S10

Protocol

- No brushing teeth, eating, drinking other than water 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 (15); c=child (21); NP=nasopharyngeal; OP=oropharyngeal; OF=oral fluid

Results with feces as other alternative specimen

Age group	Day 1				Day 15-21			
0 - <18 (n=117)	106 pairs				101 pairs			
			Feces				Feces	
			+	-			+	-
	Respiratory specimens	+	20	5	Respiratory specimens	+	5	4
		-	3	78		-	15	77
≥18 (n=123)	120 pairs				113 pairs			
			Feces				Feces	
			+	-			+	-
	Respiratory specimens	+	42	35	Respiratory specimens	+	8	17
		-	5	38		-	7	81

Added value feces and saliva at d1

Age group	Respiratory positive				Respiratory negative			
0 - <18 (n=28)	21 pairs				6 pairs			
			Feces				Feces	
			+	-			+	-
	Oral fluid	+	12	1	Oral fluid	+	0	0
		-	5	3		-	0	6
≥18 (n=17)	15 pairs				2 pairs			
			Feces				Feces	
			+	-			+	-
	Oral fluid	+	12	2	Oral fluid	+	0	0
		-	0	1		-	1	1

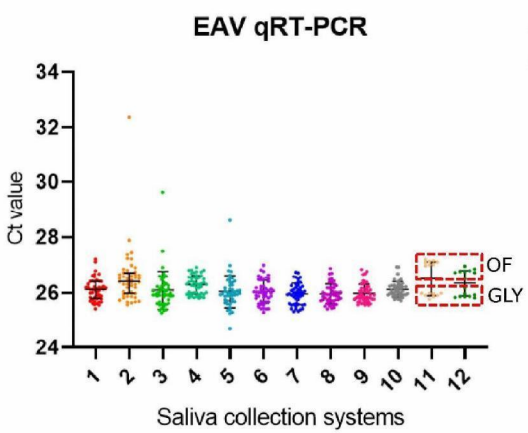
Alternative saliva collection systems

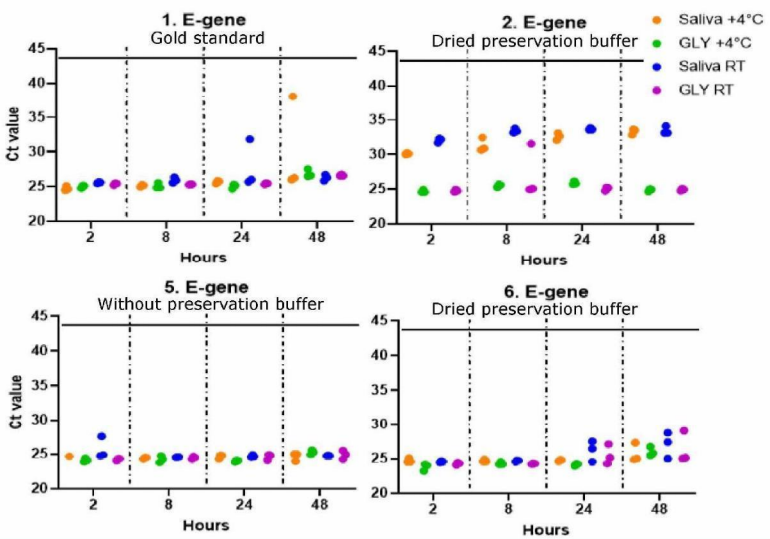
System	Manufacturer	Model	Buffer
1			
2			
3			
4			
5			
6			
7		(10)(1c)	
8			
9			
10			
11			
12			

Criteria 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

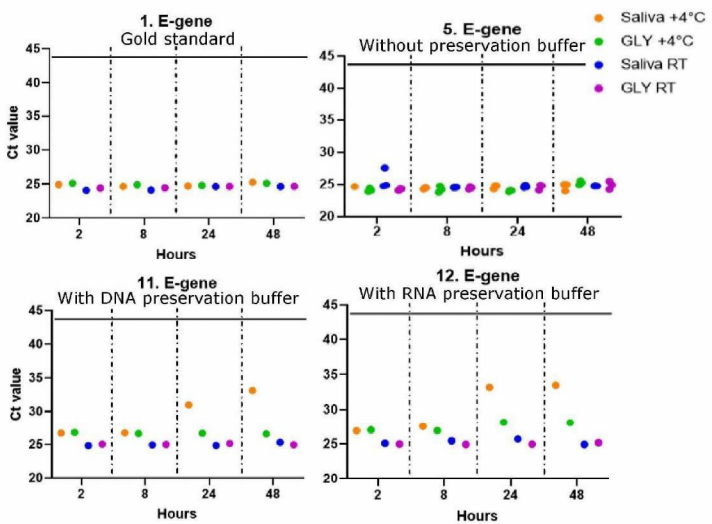




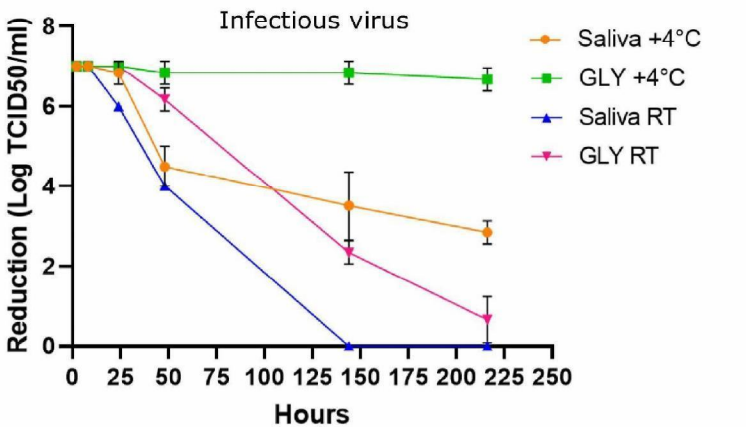
Variants Isohelix system

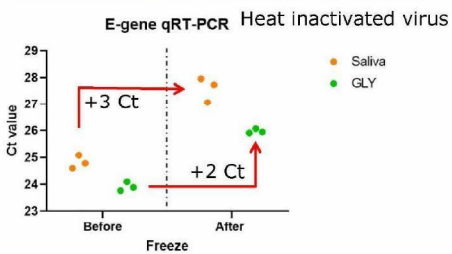
Without buffer
With DNA preservation buffer
With RNA preservation buffer

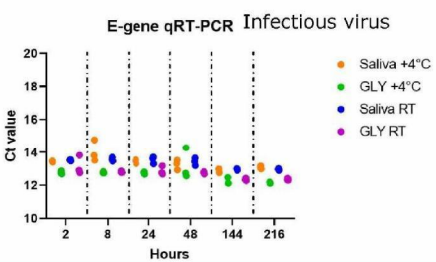
Manufacturer recommendation:
With buffer don't store at 4°C



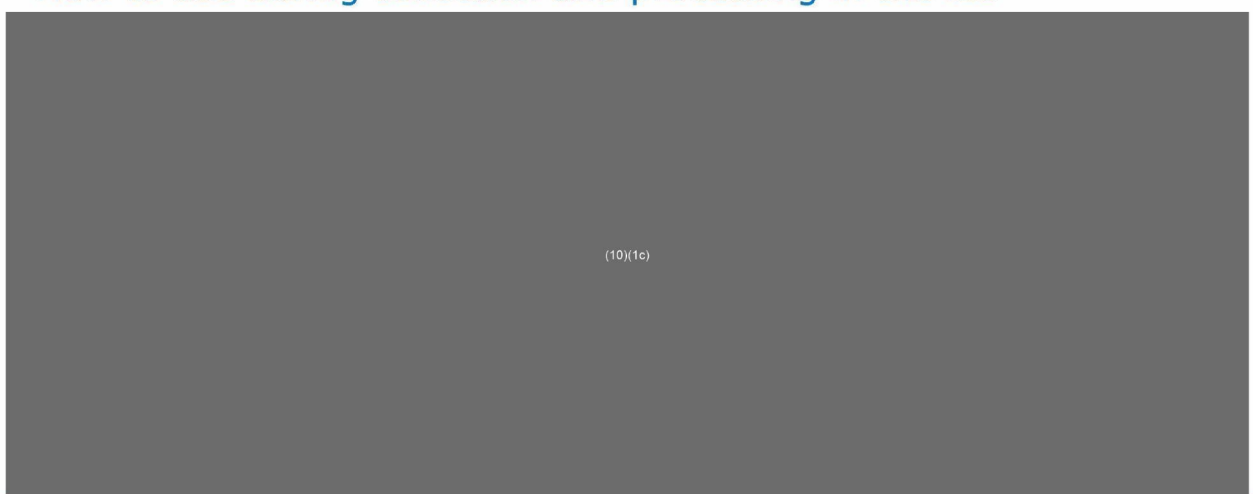
Storage oral fluid with intact SARS-CoV-2







How to use during collection and processing in the lab



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 and processing in the lab 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 cool, work quickly and do not freeze/thaw

Acknowledgements

- RIVM-IDS Virology
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