



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?

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

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

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COVID-19 SARS-CoV-2
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Subject Area

Infectious Diseases (except

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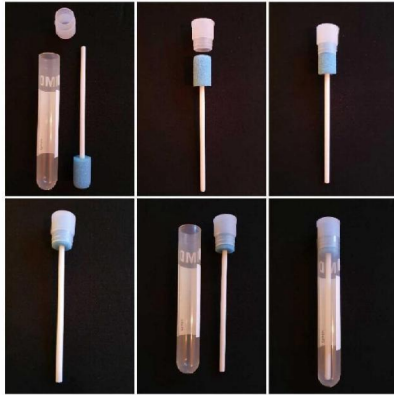


Design FFX household study

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



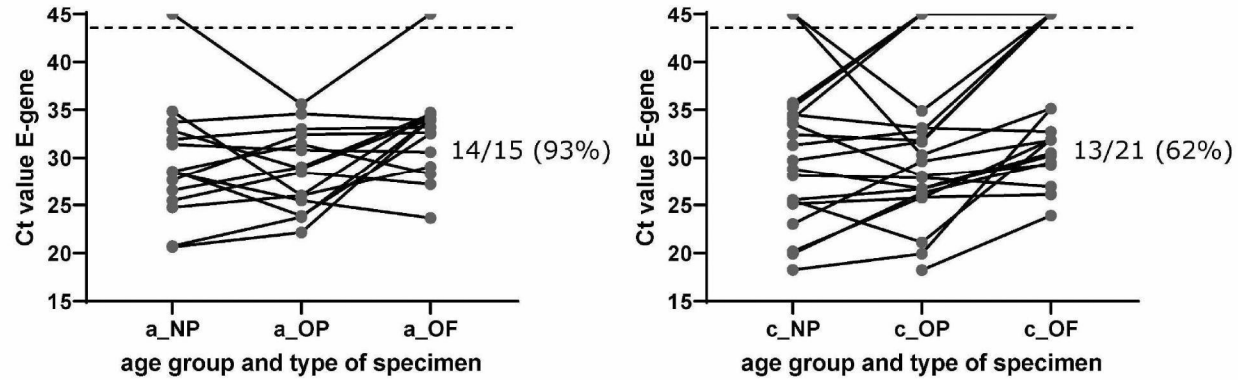
Oracol S10

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			
8			
9			
10			
11			
12			

(10)(1c)



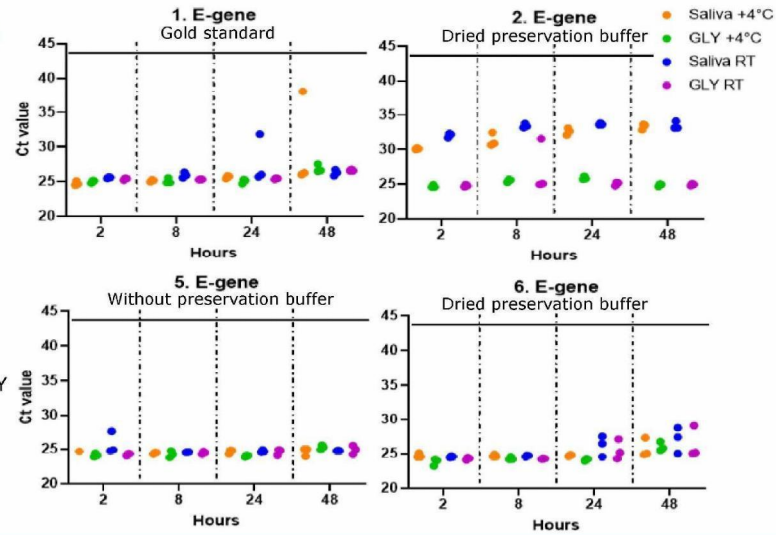
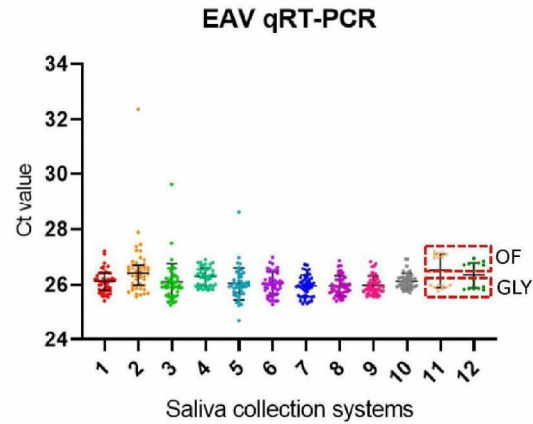


Criteria for selection

- 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



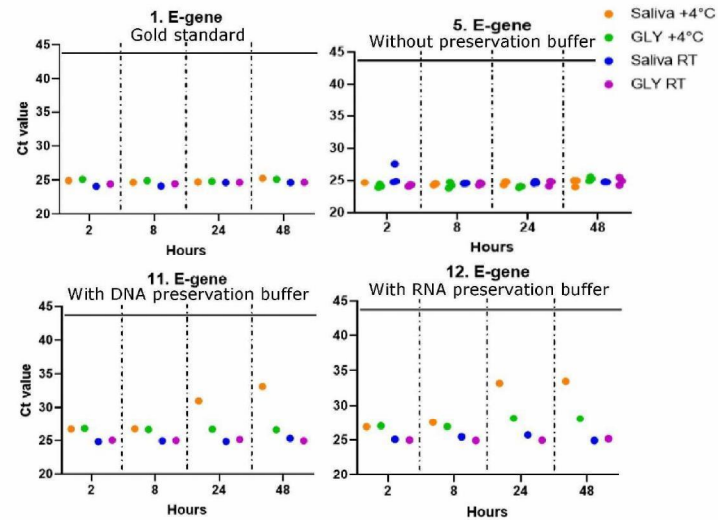
Without buffer

With DNA preservation buffer

With RNA preservation buffer

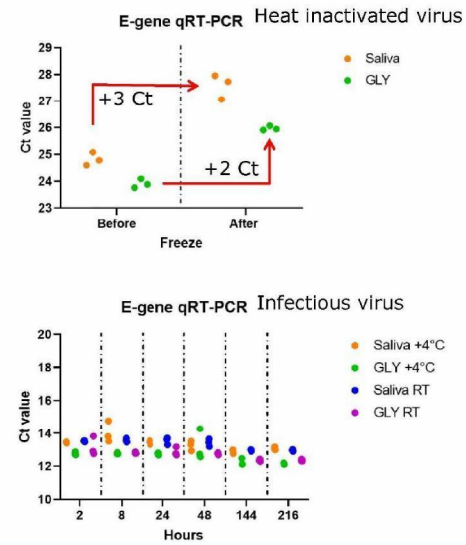
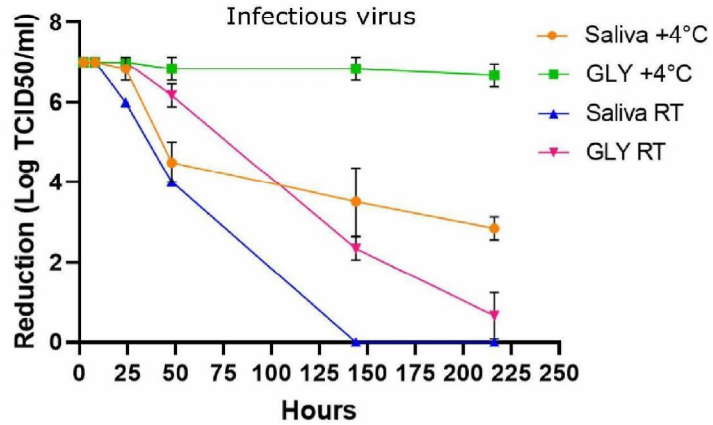
Manufacturer recommendation:

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

(10)(1c)



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 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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- RIVM-EPI

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- RIVM-IIV

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