

1 **Self-collected Saline Gargle Samples as an Alternative to Healthcare Worker Collected**
2 **Nasopharyngeal Swabs for COVID-19 Diagnosis in Outpatients**

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17 **Running title:** Performance of self-collected samples for COVID-19

18 **Key words:** COVID-19; SARS-CoV-2; gargle; saliva; mid-turbinate; PCR

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24 **Abstract**

25 **Background:** We assessed the performance, stability, and user acceptability of swab-
26 independent self-collected saliva and saline mouth rinse/gargle sample types for the
27 molecular detection of SARS-CoV-2 in adults and school-aged children. **Methods:**
28 Outpatients who had recently been diagnosed with COVID-19 or were presenting with
29 suspected COVID-19 were asked to have a nasopharyngeal swab collected and provide at
30 least one self-collected sample type. A portion of participants were also asked about sample
31 acceptability. Samples underwent molecular testing using multiple assays. Saline mouth
32 rinse/gargle and saliva samples were tested daily at time zero, day one, and day 2 to assess
33 nucleic acid stability at room temperature. **Results:** 50 participants (aged 4 to 71 years) were
34 included; of these, 40 had at least one positive sample and were included in the primary
35 sample yield analysis. Saline mouth rinse/gargle samples had a sensitivity of 98% (39/40)
36 while saliva samples had a sensitivity of 79% (26/33). Both saline mouth rinse/gargle and
37 saliva samples showed stable viral RNA detection after 2 days of room temperature storage.
38 Mouth rinse/gargle samples had the highest (mean 4.9) and HCW-collected NP swabs had the
39 lowest acceptability scores (mean 3.1). **Conclusion:** Saline mouth rinse/gargle samples
40 demonstrated the highest combined user acceptability ratings and analytical performance
41 when compared with saliva and HCW collected NP swabs. This sample type is a promising
42 swab-independent option, particularly for outpatient self-collection in adults and school aged
43 children.

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49 **Background**

50 Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has caused a
51 global pandemic that has shown the potential to overwhelm the capacity of local and national
52 health care systems (1). Rapid scale-up of molecular testing for SARS-CoV-2 has occurred
53 on an unprecedented scale and has presented numerous challenges including deployment of
54 assays with variable performance, lack of availability of high throughput testing platforms,
55 and bottle-necks in supply chain procurement of pre-analytical materials and testing reagents
56 (2). One of the key weaknesses in the diagnostic cycle has been the challenge of sample
57 acquisition. While nasopharyngeal flocked swab (NPFS) collection has been the gold-
58 standard for diagnosis of active SARS-CoV-2 infections using NAT methods (3), it is
59 difficult to collect due to the associated discomfort, and is resource intensive requiring health
60 care workers (HCW) for collection and procurement of these collection devices has proved to
61 be a challenge in many jurisdictions. This quality initiative was carried out in order to meet
62 anticipated increases in outpatient testing demands the expected challenges associated with
63 global NPFS supply chain, the difficult nature of NPFS sample collection and complex
64 handling of highly viscose saliva samples in the laboratory. We compared the analytical
65 performance, sample stability and user acceptability of saline gargle and saliva samples
66 against healthcare provider collected NPFS in out-patients with COVID-19 infections.

67

68 **Methods**

69 Individuals from the Vancouver Greater Metropolitan Area of British Columbia were
70 approached for participation in this quality-improvement initiative if they had SARS-CoV-2
71 detected in any clinical sample collected at an outpatient testing centre or if they were

72 identified as a symptomatic household contact of a confirmed COVID-19 case. After August
73 28th, 2020 symptomatic children 4 to 12 years of age presenting to the BC Children’s and
74 Women’s Hospital Campus COVID-19 Collection Centre were also asked to provide a saline
75 mouth rinse/gargle sample in addition to an NPFS. Verbal consent was obtained from all
76 participants and for those who provided both saliva and saline mouth rinse/gargle samples the
77 order of sample collection was alternated sequentially (i.e. saliva first vs mouth rinse/gargle
78 sample first). Demographic and clinical information was collected including age, whether
79 they were a health care worker (HCW), if they had been previously admitted to hospital, the
80 date of onset of their symptoms, and the date of initial molecular diagnosis of COVID-19 (if
81 applicable). Sample collection occurred either at the participant’s residence or at the BC
82 Children’s and Women’s Hospital Campus COVID-19 Collection Centre, and instruction
83 sheets (**Supplement 1**) with supporting verbal instructions were provided. Participants were
84 asked to not eat, drink, smoke, brush their teeth or chew gum 1 hour prior to collection.

85 *Self-collection procedures*

86 Nasopharyngeal flocked swabs (flexible flocked swabs with 3 mL universal viral transport
87 system media, Beckon Dickinson, Sparks, MD, USA) were collected via the left naris unless
88 preference was stated for the right naris. NPFS were inserted the distance from the naris to
89 the external ear canal and then rotated 5 times and left in place for 5 – 10 seconds prior to
90 being removed as per Center for Disease Control instructions for collection (4). Mouth
91 rinse/gargle specimens were self-collected by instructing users to open 5 ml vials of sterile
92 0.9% saline (Addipak®, Teleflex Medical, Research Triangle Park, NC, USA) and squeeze
93 the contents into their or their child’s open mouth. They were then asked to swish the
94 contents for 5 seconds followed by tilting their heads back and gargling for 5 seconds. This
95 swish/gargle cycle was repeated 2 more times and then the saline was expelled into a wide
96 mouthed sterile empty polypropylene container (Leakbuster™ 90 ml container, Starplex

97 Scientific, Etobicoke, Ontario, Canada). For saliva sample collection, participants were asked
98 to pool and spit saliva repeatedly into a wide mouthed sterile empty polypropylene container
99 (Leakbuster™ 90 ml container, Starplex Scientific, Etobicoke, Ontario, Canada) until at least
100 5 – 10 ml were collected if possible. Samples were immediately brought to the laboratory and
101 were processed within 12 hours of collection. All samples were vortexed for at least 10
102 seconds prior to aliquoting for testing.

103 *Laboratory testing*

104 All samples were extracted with the QiaSymphony automated extractor using the DSP
105 virus/pathogen minikit (Qiagen, Germantown, MD, USA) and subsequently tested with a
106 laboratory developed test (LDT) within 24 hours of collection on the Applied Biosystems
107 7500 Fast Real-Time PCR System (Life Technologies, Carlsbad, CA). Mouth rinse/gargle
108 and saliva samples were also stored in the laboratory at room temperature (21°C) and
109 extracted and tested again on day 1 and day 2 after collection. The previously validated LDT
110 is a triplex reverse transcription polymerase chain reaction (RT-PCR) assay that targets both
111 the pan-sarbecovirus E gene as well as the RNA-dependent RNA polymerase (RdRP) gene
112 and also targets the human RNase P gene as a sample positive control (5). A portion of the
113 NPFS and mouth rinse gargle specimens were also tested using the Cepheid Xpert Xpress
114 SARS-CoV-2 assay on the GeneXpert system (Cepheid, Sunnyvale, CA) as per manufacturer
115 instructions.

116 *User acceptability of sampling*

117 Participants who had all three samples collected were asked about the acceptability of each
118 self-collected sample type they had obtained as well as about the HCW-collected NPFS using
119 a 5 point Likert scale with 1 being “least acceptable” and 5 being “most acceptable”.

120 This project was reviewed by the BC Children’s and Women’s Research Ethics Board and
121 deemed a quality improvement/quality assurance (QI/QA) activity based on completion of
122 the Provincial Health Services Authority Project Sorting Tool.

123 *Statistical analysis*

124 For the purpose of the analysis of sensitivity for each sample type, individuals were
125 considered a positive case if they had at least one sample test positive for both targets (E gene
126 and RdRP gene) on the LDT. Samples were considered positive with a threshold cycle (Ct)
127 value of less than 40 on the LDT and as reported by the Xpert assay (presumptive positive
128 results were considered as positive). Using this reference standard for a SARS-CoV-2
129 positive participant the sensitivity results of mouth rinse/gargle sampling was compared to
130 NPFS and saliva sampling (LDT assay) using the test for comparing two independent
131 proportions (STATA command `prtesti`); the McNemar exact test was also used to determine
132 the comparability of mouth rinse/gargle sample testing as compared to NPFS or saliva sample
133 testing. The Ct values obtained with baseline testing using all sample types were compared
134 using repeated-measures ANOVA using Box’s conservative correction factor, with *post hoc*
135 significant pairwise differences determined using Tukey’s HSD; this methodology was also
136 used to compare acceptability ratings of all sample types. For assessment of stability of
137 SARS-CoV-2 RNA between gargle samples and saliva samples (transport media free
138 methods), individuals were only included if they had samples tested at each of those three
139 time points (time zero, day 1, and day 2). Statistical significance was set as $p < 0.05$. All
140 testing was done using STATA v16.1 (College Station, TX).

141

142 **Results:**

143 A total of 50 participants (34 known positives and 16 household
144 contacts/symptomatic children) provided validation samples between May 8th and September
145 11th, 2020. Of these, 40 participants were found to be positive according to the reference
146 definition at the time of study sample collection. There were 28 (56%) participants that were
147 female, with a median age of 25.1 years (25-75 percentile 13.6-35.9 years) and 10 were < 18
148 years of age. Data on symptoms was available for 40 participants; of these, 17 (42%) had
149 fever and 25 (62%) had cough. Among participants found to be positive, the median time
150 from infection confirmation to re-testing was 3 days (25-75 percentile 2-5 days) and the
151 median time from symptoms to re-testing was 7 days (25-75 percentile 4-9 days).

152 The results of testing for each of the sample types for all positive participants are
153 shown in **Table 1**. Mouth rinse/gargle samples were significantly more likely to be positive
154 than saliva samples (difference of 18.7%, 95%CI 3.9-33.5%, p=0.01). When matched
155 samples were compared, mouth rinse/gargle sample testing results differed statistically from
156 saliva samples (26 positive by both, 6 rinse/gargle positive but saliva negative, and 1 negative
157 by both, McNemar p=0.03) but mouth rinse/gargle sample and saliva testing results were
158 statistically similar to HCW-collected NPFS testing results. Positivity proportions for NPFS,
159 mouth rinse/gargle and saliva sample types based on time from COVID-19 diagnosis are
160 shown in **Figure 1**.

161 Participant rated acceptability performance of sample types is shown in **Table 2**. There were
162 clear differences between the acceptability of the sample types; the mouth rinse/gargle
163 sample had the highest mean acceptability (4.95) and was significantly more acceptable than
164 HCW-collected NPFS (mean acceptability 3.17) or saliva sampling (mean acceptability
165 4.44).

166 Mean Ct values at baseline did not differ significantly between sample types; these data are
167 shown in **Figure 2**. Mean Ct data at day zero, day 1, and day 2 for mouth rinse/gargle and
168 saliva samples are shown in **Figure 3**. There was no significant difference in baseline Ct
169 values across sample types, and for saliva and mouth rinse/gargle samples there was no
170 significant loss of RNA recovery over all time points. Also amongst positive participants with
171 mouth rinse/gargle (n = 30) and saliva (n = 28) samples that were tested on all three days
172 there were a similar number of negative results (for both targets) on each day (saliva day 0=
173 6, day 1 = 4 and day 2 = 3; mouth rinse/gargle day 0 = 1, day 1 = 5, and day 2 = 2).

174

175 **Discussion:**

176 Laboratory diagnostics are critical for any attempt to contain the COVID-19 pandemic. Our
177 study demonstrates that self-collected mouth rinse/gargle samples are non-inferior to HCW-
178 collected NPFS for the detection of SARS-CoV-2 and, at the same time, are significantly
179 more acceptable to patients. Mouth rinse/gargle specimens, in particular, demonstrated the
180 highest sensitivity and were preferred by those undergoing sampling. These data should be
181 considered by all those planning laboratory testing strategies and algorithms; self-collected
182 mouth rinse/gargle samples may obviate the need for the deployment of significant numbers
183 of HCWs trained in sample collection and the consumption of large amounts of personal
184 protective equipment. It should be emphasized that testing volumes will very likely be even
185 higher in late 2020 as many children and adolescents return to school and respiratory
186 symptoms become more common with the increasing prevalence of many other respiratory
187 viruses.

188 Saline mouth rinse/gargle samples (also known as mouth throat washes) have previously
189 been evaluated for influenza and other respiratory virus detection (6, 7), showing promising

190 performance when compared to throat swab and other sample types. Interestingly, although
191 there have been numerous evaluations of multiple self-collected sample types including mid-
192 turbinate swabs, throat swabs, and saliva samples, there have been very few reports to date
193 describing mouth rinse/gargle samples for COVID-19 diagnosis (8, 9). In one study in
194 Germany (10), 5 individuals were evaluated who had both saline gargle samples and throat
195 swab samples and all 5 gargle samples were positive whereas only 4 had a positive throat
196 swab sample. The technique we used for acquisition of saline mouth rinse/gargle samples
197 (three cycles for mouth rinse followed by gargle) may allow for improved recovery of viral
198 RNA given that it would effectively sample the entire oropharynx. Collecting mouth
199 rinse/gargle samples could also potentially result in a significant amount of savings due to the
200 lack of need for personal protective equipment or trained HCWs for sample acquisition. Its
201 utility might be even higher in low and middle income country settings or remote regions
202 where access to testing clinics would be another barrier to sample acquisition. The stability of
203 RNA recovery in this sample type was preserved for at least two days at room temperature,
204 making later drop-off delivery of samples a feasible option.

205 In our study, saliva samples were both significantly less sensitive and less acceptable than
206 mouth rinse/gargle samples. Several recent reports have found favourable detection rates for
207 saliva samples, however most of these have been in inpatient populations (11) where viral
208 load levels tend to be higher and/or utilized additional manual saliva processing steps (12)
209 that are less amenable to high throughput processing. In our evaluation processing of saliva
210 samples was intermittently observed to be impeded by variable sample viscosity; additional
211 manual processing of two saliva samples was required due to excessive amounts of mucus
212 present. For these reasons, amongst the swab- and transport media free options, the mouth
213 rinse/gargle samples appeared more attractive.

214 Our study design had several strengths. In addition to having a number of participants across
215 the pediatric and adult age ranges, we also enrolled solely individuals presenting with
216 outpatient illness, where the largest burden of testing is performed. This is particularly the
217 case for testing surges that are expected with return to school for children in the fall. Our
218 evaluation also includes the assessment of performance across multiple extraction and PCR
219 platforms, including a Health Canada and FDA authorized commercial assay. A main
220 weakness of the study is that a number of the patients enrolled did not have samples collected
221 until > 5 days into their illness and therefore we had a number of samples with lower amounts
222 of viral RNA detected. Also as mentioned, the population sampled only included relatively
223 well outpatients and it is unclear if similar performance would be found in those with more
224 severe inpatient disease.

225 Given the very high user acceptability rating, lack of need for swabs and/or transport media,
226 and excellent diagnostic yield, saline mouth rinse/gargle samples appear to be a preferred
227 sample type for testing of outpatients with suspected COVID-19.

228 **Funding**

229 No external funding was provided for this project.

230 **Conflicts of interest**

231 We have no conflicts of interest to disclose.

232 **Acknowledgements**

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Table 1. Performance of sample types in 40 COVID-19 positive participants

Study ID	NP Swab (LDT)	NP Swab (Gx)	Gargle (LDT)	Gargle (Gx)	Saliva (LDT)
1	P	P	P	P	P
2	P	P	P	P	N
3	N	PP	P	NT	N
4	P	P	P	PP	N
5	P	P	P	P	P
6	NT	NT	P	P	P
7	P	P	P	P	P
8	P	P	P	P	P
9	P	P	P	P	P
10	P	P	P	P	N
11	P	P	P	P	P
12	P	P	P	P	P
13	P	P	P	P	P
14	P	P	P	P	P
15	P	P	P	P	P
16	P	P	P	P	P
17	P	P	P	P	P
18	P	P	P	P	P
19	P	P	P	P	P
20	NT	NT	P	P	P
21	NT	NT	P	P	P
22	P	P	P	P	P
23	P	P	P	P	P
24	P	P	P	P	N
25	P	P	P	P	P
26	P	P	P	P	P
27	P	P	P	P	P
28	N	N	P	P	P
29	P	P	P	P	P
30	P	P	P	N	P
31	P	P	N	P	N
32	P	P	P	P	N
33	P	P	P	P	P
34	P	NT	P	P	NT
35	P	NT	P	P	NT
36	P	NT	P	P	NT
37	P	NT	P	P	NT
38	P	NT	P	P	NT
39	P	NT	P	P	NT
40	P	NT	P	P	NT
N=40	35/37	29/30	39/40	38/39	26/33
Sensitivity	NA	NA	97.5%	97.4%	78.8%
95% CI	NA	NA	86.8, 99.9	86.5, 99.9	61.0, 91.0

NP = nasopharyngeal, LDT = laboratory developed test, Gx = genexpert assay, NA = not applicable, P = positive test, N = negative test, PP = presumptive positive, NT = not tested, CI = confidence interval

287 **Table 2. Acceptability of sample types as rated on a 5 point Likert scale (1= lowest**
288 **acceptability and 5 = highest acceptability)**

Sample	Mean acceptability	Acceptability difference		95%CI of difference	Tukey p of difference
Mouth Rinse/Gargle	4.95	vs: saliva	0.50	0.0074 to 0.99	0.046
		NPFS	1.78	1.28 to 2.27	<0.001
Saliva	4.44	vs: NPFS	1.28	0.78 to 1.77	<0.001
NPFS	3.17				

289 NPFS = nasopharyngeal flocculated swab, CI = confidence interval

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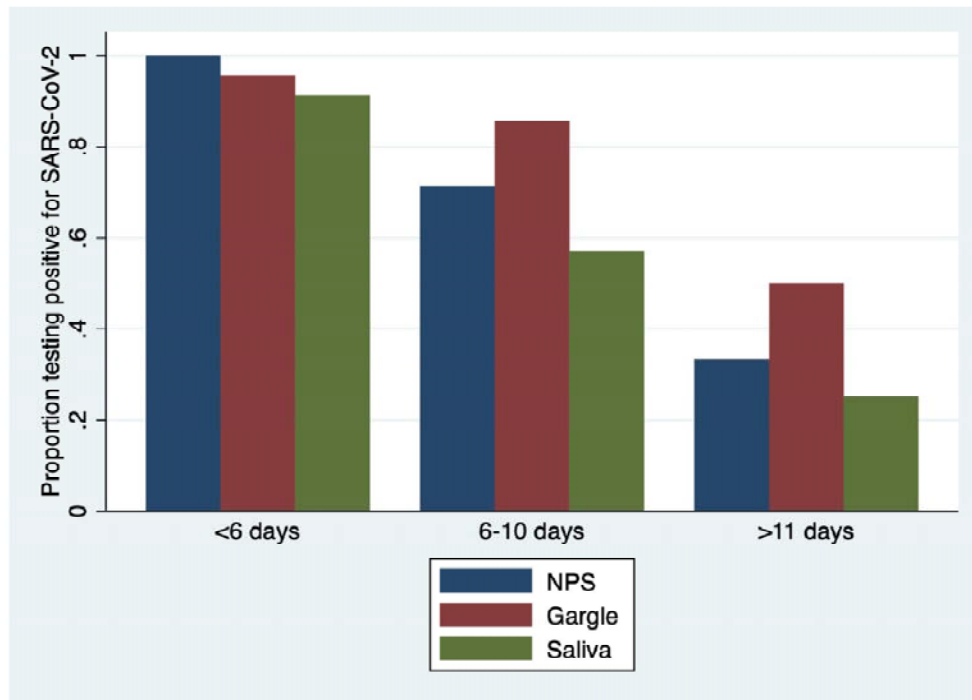
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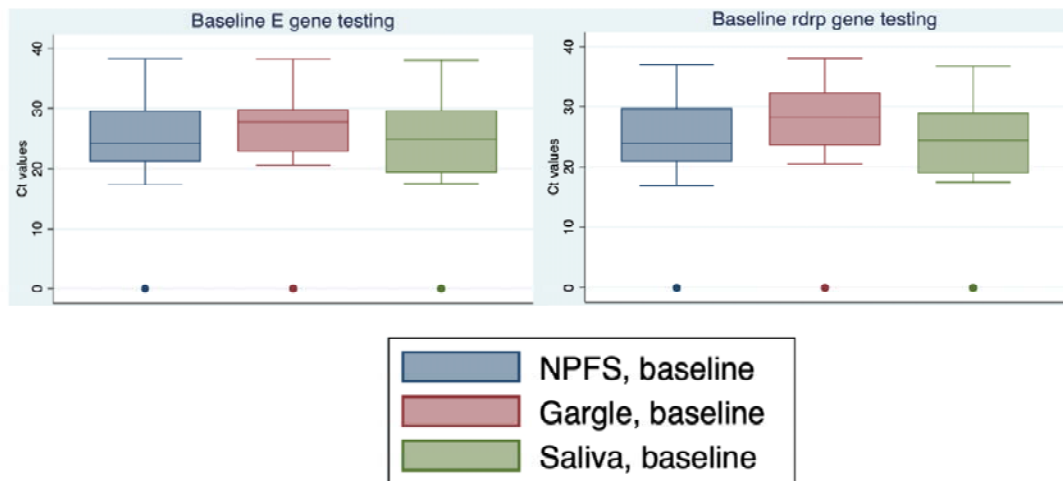
Figure 1. Proportion of samples testing positive based on time from COVID-19 diagnosis



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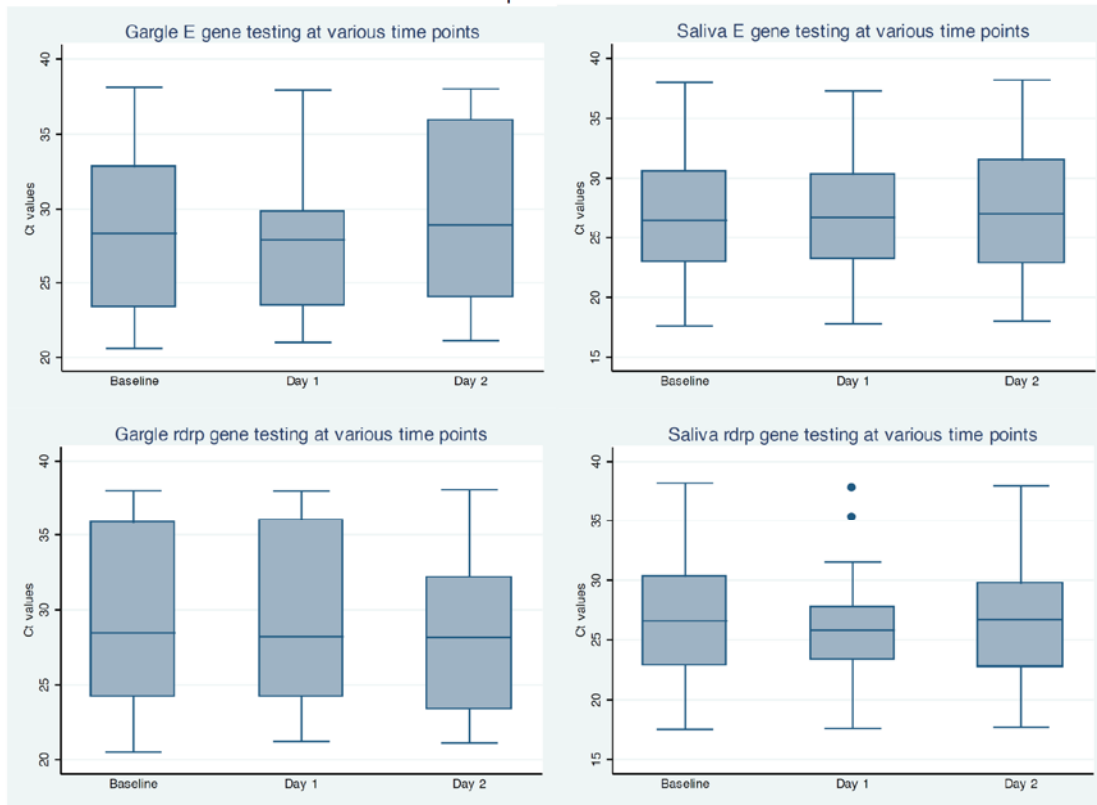
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Figure 2: Mean threshold cycle (Ct) values across sample types at baseline



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Figure 3. Mean threshold cycle (Ct) values at baseline, Day 1, and Day 2 for gargle and saliva samples with positive results



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