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## Testing individual and pooled saliva samples for sars-cov-2 nucleic acid: a prospective study

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1 **Title:**

2 Testing individual and pooled saliva samples for sars-cov-2 nucleic acid: a prospective study

3

4 **Running Title:**

5 Individual and pooled saliva to detect SARS-CoV-2

6

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28 **Highlights**

29

30 • Efficient testing is required to control the rapid spread of the SARS-CoV-2 virus

31 • We conducted a prospective study assessing individual and pooled saliva sensitivity

32 • Saliva-based tests are sensitive and may increase patient adherence with screening

33 • Mass screening of pools of five specimens is a strategy worth considering

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40 **Abstract (138 words)**

41 Control of the rapid spread of the SARS-CoV-2 virus requires efficient testing. We collected  
42 paired nasopharyngeal swab (NPs) and saliva samples from 303 subjects (52.8%  
43 symptomatic) at a drive-through testing center; 18% of whom tested positive. The NPs,  
44 salivas and five saliva pools were tested for SARS-CoV-2 RNA using the Aptima™ assay  
45 and a laboratory-developed test (LDT) on the Panther-Fusion™ Hologic® platform. The  
46 saliva sensitivity was 80% (LDT) and 87.5% (Aptima™) whereas that of NPs was 96.4% in  
47 both assays. The pooled saliva sensitivity of 72.7% (LDT) and 75% (Aptima™) was not  
48 significantly different of that of individual saliva testing. Saliva specimens appear to be  
49 suitable for sensitive non-invasive assays to detect SARS-CoV-2 nucleic acid; pooling them  
50 for a single test will improve laboratory throughput.

51

52 **Keywords**

53 SARS-CoV-2, saliva, nasopharyngeal swabs, pooling, COVID-19, RT-PCR, TMA

54

55

56

## 57 **Introduction**

58           The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected over  
59 175 million individuals causing more than 3.8 million of deaths as of June 24, 2021 (1). Many  
60 governments have used various testing strategies to contain the pandemic and reduce the  
61 spread of the virus. The gold standard test assays SARS-CoV-2 RNA in nasopharyngeal  
62 swabs (NPs) (2,3). However, the NPs samples are obtained using invasive method that  
63 requires trained healthcare personnel and suitable protective equipment. Many patients find it  
64 uncomfortable, which dissuades others and reduces compliance.

65           Saliva sampling is non-invasive, painless and much more acceptable. It does not use  
66 swabs, and patients can easily collect their own samples, so reducing crowding at testing sites.  
67 However, the results of studies that used saliva samples for SARS-CoV-2 diagnosis have  
68 been variable. Some reported that saliva samples were inappropriate (4–6), while others found  
69 them acceptable or even provided greater sensitivity (7–13). These differences could be due  
70 to the sampling protocol used (saline gargle and spit, posterior oral saliva, crude saliva, oral  
71 swabs) or the individuals tested (hospitalized patients or health-care workers in most cases).  
72 Few studies assessed the performance of saliva samples for SARS-CoV-2 testing according to  
73 the time from symptom onset, or in asymptomatic non-hospitalized individuals (10,14–16). In  
74 addition, little is known of the suitability of pooled saliva samples for increasing laboratory  
75 throughput, which optimizes reagent use and reduces cost.

76           This prospective study of outpatients examined SARS-CoV-2 detection in individual  
77 and pooled saliva specimens, and NP swabs. SARS-CoV-2 RNA was detected using two  
78 assays: the Aptima<sup>TM</sup> SARS-CoV-2 transcription-mediated amplification (TMA) (Aptima<sup>TM</sup>)  
79 and a laboratory-developed test (LDT) that uses RT-PCR on the Panther Fusion<sup>TM</sup> Hologic®  
80 platform.

81

## 82 **Materials and methods**

### 83 **Study Subjects**

84 Patients who came to the Toulouse University Hospital drive-through testing center 27  
85 - 29 October 2020 during the second epidemic wave in France were enrolled prospectively.  
86 They were informed of and consented to the test before they provided a saliva specimen plus  
87 a NP swab sample. The saliva samples were self-collected under the supervision of a health  
88 care worker (HCW) and the NPs was collected by a HCW following CDC specimen  
89 collection guidelines (17).

90

### 91 **Specimen Collection and Processing**

92 NPs were collected on flocked swabs and placed in 3.5mL of virus transport medium  
93 (VTM) (Virus sampling kit, Yocon, Beijing, China). Saliva (1mL) was collected after the  
94 subjects had swilled their saliva around their mouths for at least 30s and then spitting into a  
95 sterile container (12). The saliva samples were diluted 3-fold dilution in Minimum Essential  
96 Media (MEM) and all samples, NPs and saliva, were tested in the clinical laboratory within  
97 24 hours.

98 Pooled saliva (5 sequential individual samples) and individual patient salivas were  
99 used to evaluate pooling.

100

### 101 **Laboratory testing**

102 All specimens (NPs, individual and pooled salivas) were tested simultaneously. We  
103 used the Aptima™ SARS-CoV-2 transcription mediated amplification assay (TMA)  
104 (Hologic®, San Diego, California) and a laboratory-developed test (LDT) based on real-time  
105 RT-PCR on a Panther Fusion™ module (Hologic®, San Diego, California) that has been  
106 validated on saliva specimens (12). The Aptima™ assay targets two sequences on the virus

107 ORF1ab gene. The LDT targets two sequences on the virus RNA-dependent RNA-  
108 polymerase (RdRp) gene (IP2, IP4, Institut Pasteur, Paris, France) (18). An internal control  
109 was included in both assays.

110 Sample processing was the same for both methods. 500  $\mu$ L of VTM for NPs or 500 $\mu$ L  
111 of diluted saliva were placed in Aptima lysis tubes (Hologic®, San Diego, California). Saliva  
112 pools were prepared by mixing 100 $\mu$ L diluted saliva from each of five subjects directly in an  
113 Aptima lysis tube.

114 Samples with invalid result by either assay were re-tested after adding 500 $\mu$ L MEM directly  
115 to the Aptima lysis tube.

116

#### 117 **Statistical analysis:**

118 We calculated the sensitivity of NPs and saliva by using the total number of positive  
119 patients diagnosed by either test as the reference standard. The sensitivity differences between  
120 individual and pooled saliva were calculated by dividing the number of patients missed with  
121 pooling compared to individual treatment by the number of positive patients diagnosed with  
122 saliva. The 95% confidence intervals (CI 95%) were calculated by the Clopper and Pearson  
123 method using GraphPad Prism. The saliva sensitivity between asymptomatic and  
124 symptomatic individuals was compared using Fisher's exact tests. Pooled saliva sensitivity  
125 and individual saliva sensitivity were compared using Fisher's exact tests. Statistically  
126 significant difference was defined as  $P < 0.05$ .

127 Cycle threshold (Ct) values for saliva and NPs samples were compared using  
128 Student's paired t-test. The Ct values of NPs from individual groups (symptomatic and  
129 asymptomatic, or paired NPs and saliva from positive individuals and only NPs from positive  
130 individuals) were compared using the Mann-Whitney U-test.

131

## 132 **Results**

133 A total of 606 NPs and saliva samples were collected from 303 subjects (average age:  
134 33 years; range: 03 - 77 years; 52.5% female). The 303 subjects included 160 (52.8%) who  
135 were mildly symptomatic and 143 (47.2%) who were asymptomatic at the time of sampling.  
136 Two saliva samples gave invalid results even after additional dilution and repeated testing  
137 with both assays. Thus, 301 paired NPs-saliva samples were analysed. We tested 61 saliva  
138 pools, 60 pools of five samples and one of three.

139

### 140 1. Detection of SARS-CoV-2 RNA in individual specimens with the LDT

141 Overall, 55 subjects tested positive using NPs or saliva specimens and 42 tested positive  
142 with both samples (Table 1). Global sensitivity of saliva and NPs were 80% [95% CI: 67.0%-  
143 89.6%] and 96.4% [95% CI: 87.5%-99.6%] respectively. The mean difference between paired  
144 positive NPs/saliva samples using the IP2 target was 8 Ct [95% CI: 6.57-9.43] and that  
145 between these samples on the IP4 target was 8.2 Ct [95% CI: 6.47-9.99] ( $p < 0.001$ ) (Figure 1).

146 The 55 COVID-19 positive patients included 9 who were asymptomatic and 46 who were  
147 mildly symptomatic. Of the symptomatic patients, 41 were tested during the first week of  
148 symptoms and five were tested later. We found a higher saliva sensitivity for symptomatic  
149 people (84.8% [95% CI: 71.1%-93.7%]) than for asymptomatic ones (55.6% [95% CI:  
150 21.2%-86.3%]) (Table 2). The median Ct values for NPs from asymptomatic individuals were  
151 significantly higher (IP2 Ct: 35.6; IP4 Ct: 33.8) than those for symptomatic subjects (IP2 Ct:  
152 24.9, IP4 Ct: 22.7) ( $p = 0.019$  (IP2) and  $p = 0.009$  (IP4)) (Figure 2). The median Ct values for  
153 patients whose NP was positive and their saliva was negative (IP2 Ct: 36.3 and IP4 Ct: 33.6)  
154 were higher than those of patients whose NP and saliva were positive (IP2 Ct: 24.6 and IP4  
155 Ct: 20.9) ( $p < 0.001$  and  $p < 0.001$ ) (Figure 3).

156



157 2. SARS-CoV-2 RNA detection in individual samples with the Aptima™ assay

158 The NPs or saliva samples from 56 subjects tested positive, and both saliva and NP  
159 samples from 47 subjects tested positive (Table 3). Saliva and NPs sensitivity were 87.5%  
160 [95% CI: 75.9%-94.8%] and 96.4% [95% CI: 87.7%-99.6%] respectively. Among these 56  
161 COVID-19 positive patients, 10 were asymptomatic and 46 mildly symptomatic. Saliva  
162 sensitivity was higher for symptomatic people (93.5% [95% CI: 82.1%-98.6%]) than for  
163 asymptomatic ones (60% [95% CI: 26.2%-87.8%]) (p=0.015) (Table 2).

164

165 3. SARS-CoV-2 RNA detection in pooled saliva samples

166 The 61 saliva pools included 31 that tested positive and 30 that tested negative in the LDT  
167 assay. Among the 31 positive pools, 28 included at least one positive specimen looking at  
168 individual saliva results: 18 with only 1 positive sample, 8 pools with 2 positive samples and  
169 2 pools with 3 positive samples. Therefore, 40 subjects were diagnosed using the pooling  
170 strategy giving an overall sensitivity of 72.7% [95% CI: 59.0%-83.9%] not significantly  
171 different of that of individual testing (p=0.5) (Table 4). With 4 patients missed with this  
172 strategy compared to individual saliva testing, we observed a loss of sensitivity of 9.1% [95%  
173 CI: 2.5%-21.7%]. Three pools tested positive whereas the individual saliva results with the  
174 LDT assay were all negative. These three pools all contained a single saliva specimen that  
175 tested positive only in the Aptima™ assay and whose paired NPs tested positive in both  
176 assay. Potential RT-PCR inhibitors that would have been diluted thanks to the pooling  
177 strategy could explain the negative result of these saliva samples in individual treatment with  
178 the LDT assay.

179

180 With the Aptima™ assay, 29/61 pools tested positive. The 29 positive pools included 18  
181 with only 1 positive sample, 9 pools with 2 positive samples, and 2 pools with 3 positive

182 samples. Thus, with 42 subjects detected the overall sensitivity of pooled saliva of 75.0%  
183 [95% CI: 61.6%- 85.6%] was not significantly different of that of individual saliva (p= 0.15).  
184 The loss of sensitivity compared to individual saliva testing was 14.3% [95% CI: 5.9%-  
185 27.2%] with 7 patients missed (Table 5).

186

## 187 **Discussion**

188 The emergence of new more infective strains of virus has made it essential that the  
189 SARS-CoV-2 screening strategies should be as effective as possible (19) . Sample pooling  
190 seems to be promising strategy (20) but very few data are available on tests using saliva  
191 samples and their sensitivity is still debated. The results of this prospective study provide  
192 further evidence that non-invasive saliva sampling is an acceptable alternative to NPs. They  
193 effectively identified individuals who were most likely to spread the infection. Moreover,  
194 pooling saliva samples may increase laboratory capacity while maintaining a correct  
195 sensitivity.

196 The literature on the sensitivity of tests on saliva has not produced unanimous results.  
197 Reported sensitivities range from 46 to 100% (4,5,7,9–14,16,21–29). We find overall  
198 sensitivities of 80.0% using our LDT and 87.5% with the Aptima assay, indicating that testing  
199 saliva is acceptable for SARS-CoV-2 diagnosis in outpatients, which is consistent with the  
200 findings of a recent meta-analysis (30). The published discrepancies could be due to  
201 differences between the populations studied, the time of sampling and the patients' virus load,  
202 the saliva collection method, or even the amplification assay used. While some studies found  
203 higher virus loads in saliva than in NPs (7) (10), we and others find higher Ct values in saliva,  
204 indicating lower virus loads in saliva (5,9,12,13). This could be due to differences in the  
205 saliva collection method. A recent meta-analysis reported that tests using posterior

206 oropharyngeal saliva samples were more sensitive than samples obtained by the swill-and-spit  
207 method (30).

208         We tested saliva samples provided by subjects, both symptomatic and asymptomatic,  
209 who came to a drive-through testing center during the second epidemic wave in France,  
210 unlike the majority of studies which were done on inpatients or asymptomatic individuals. We  
211 find that tests on samples from asymptomatic individuals are less sensitive than are tests on  
212 samples from symptomatic ones, as reported by Nacher et al (15). However, others have  
213 reported that tests for SARS-CoV-2 using saliva from asymptomatic individuals gave good  
214 results (7,10,16), with no relationship between test sensitivity and symptoms (14,30). Our  
215 finding of lower sensitivity is probably due to the lower virus load in asymptomatic patients  
216 (only one asymptomatic patient had an IP2 Ct < 30) and SARS-CoV-2 infections were less  
217 common (7%) than in symptomatic ones (28%). The majority of patients who tested positive  
218 only for their NPs had a high Ct (IP2 Ct >30), which confirms previous studies showing that  
219 saliva sensitivity depends mainly on the patient's virus load and sampling time after infection  
220 (4,12), as the virus concentration in the saliva declines more quickly than that in the NP  
221 cavity (14,24). It is now well accepted that patients who have a high Ct (24-35, depending on  
222 the study) are not infectious or at least less so (31–33). Overall our results indicate that  
223 SARS-CoV-2 molecular diagnosis on saliva samples is sensitive enough to identify  
224 individuals most likely to spread the virus.

225         We observed a slight loss of sensitivity with saliva samples compared to NPS.  
226 Whereas this slight loss can be unimportant if prevalence is 0.1% (few missed cases per 1000  
227 tested) it can be very important if prevalence is 10% (many missed cases per 1000 tested).  
228 However we think, given our results, that these missed cases with saliva samples, are less  
229 likely to participate to the viral spread of the SARS-CoV-2 due to their low viral loads.  
230 Moreover, any risk that this sampling is less sensitive than NPs sampling is offset by the

231 possibility of repeated tests greatly facilitated by the non-invasiveness, the ease of collection,  
232 and the low cost of this sampling (reduced material and human resources needed) (30).

233         Several studies have shown that pooling of nasopharyngeal samples is efficient and  
234 sensitive (34–38) but few have evaluated saliva pooling (39–41). Our pools of 5 saliva  
235 samples provided data that agrees well with those obtained from individual saliva samples  
236 with both of the assays used. There was a slight loss of sensitivity due to the pooling itself  
237 (40). The pooling strategy missed four or seven patients (depending on the assay), but they  
238 were detected after testing individual salivas. This was undoubtedly due to their low virus  
239 loads (IP2 Ct > 30). In contrast, three samples tested positive in the pooled samples but were  
240 negative in individual saliva tests. This suggests that some samples should be diluted to  
241 reduce the influence of any saliva RT-PCR inhibitors (42). Overall, the slight loss of  
242 sensitivity using pooled samples rather than individual tests might be acceptable as it  
243 increases laboratory capacity for mass screening and reduces costs.

244         Most studies assessed the sensitivity of saliva samples using RT-PCR or Reverse  
245 Transcription Loop-mediated Isothermal Amplification (RT-LAMP) (16,29); only one used a  
246 TMA assay on saliva samples (27). Our results demonstrate that the Aptima™ assay is at least  
247 as efficient as a classic RT-PCR assay for detecting SARS-CoV-2 in saliva samples. The  
248 overall sensitivities were 80% with the LDT and 87.5% with the Aptima assay and the  
249 concordance between the two assays was very good with only 10/606 discordant results for  
250 individual samples.

251         This study has several limitations. Subjects only self-collected their saliva with the  
252 guidance of a health care worker. It would be informative to evaluate unsupervised saliva  
253 self-collection by subjects at home; if successful this would increase testing accessibility. This  
254 sampling method could also reduce the human resources manning testing centers. These

255 professionals could profitably be employed at laboratory testing platforms or COVID  
256 vaccination centers.

257           We evaluated pools of five saliva samples from subjects at high risk of infection  
258 (symptomatic subjects or contact cases) to obtain enough positive subjects to assess pooled  
259 saliva sensitivity. This pooling strategy is probably more suitable for mass screening, such as  
260 in a city-wide survey of mainly asymptomatic individuals and a low prevalence. The pooling  
261 of five saliva specimens was effective with an average 30% reduction in testing, despite the  
262 relatively high prevalence (18%) and high proportion of positive pools in our study. This  
263 strategy would be even more cost effective if it were used after lockdown, when prevalence is  
264 low. For example, at a prevalence of 1% or 5%, pooling 5:1 reduces at least (in the worst  
265 scenario where each positive are in different pools) the number of samples run per 100  
266 specimens from 100 to 25 or 45 respectively. While pooling is efficient to reduce reagent  
267 consuming, its implementation requires good organization with critical steps such as pooling  
268 process, tracking positive samples for individual treatment, and results reporting that could be  
269 time consuming for technicians and require an automation.

270

271           In conclusion, we believe that the good sensitivity of saliva-based tests for SARS-CoV-2  
272 nucleic acid indicates that this medium is a desirable non-invasive way of obtaining samples  
273 that is well accepted by patients and allows repeated testing. Pools of five saliva samples  
274 appear to be suitable for mass screening.

275

276

277 **CRedit author statement**

278 M Miguères and C Vellas: Investigation, Methodology, Writing - Original Draft, Statistical  
279 analysis.

280 J Izopet: Conceptualization, Methodology, Supervision, Writing - Review & Editing.

281 JM Mansuy, F Abravanel and S Raymond: Supervision and Writing – Review.

282 C Dimeglio: Statistical analysis – Review.

283 I Da Silva and V Ferrer: Methodology and Data acquisition.

284 All authors edited and approved the final manuscript.

285

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290 **Disclaimers**

291 The authors declare no conflict of interest. The funders had no role in the design of the study,  
292 in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the  
293 decision to publish the results.

294

295

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- 434
- 435

436 **Table 1:** Qualitative results for saliva and NPs using the LDT assay

437

		Saliva		
		Positive	Negative	$\Sigma$
NPs	Positive	42	11	53
	Negative	2	246	248
	$\Sigma$	44	257	301*

\*2 specimens gave invalid result

438

439

440

441

442 **Table 2:** Qualitative results for saliva and NPs with both assays according to patient's  
 443 category

444  
 445

	Saliva +ve/ NP +ve patients	Saliva +ve/NP- ve patients	Saliva -ve/ NP+ve patients	Saliva -ve/NP- ve patients
<b>LDT assay (N=301*)</b>				
<b>Asymptomatic (N=141*)</b>	4	1	4	132
<b>Symptomatic (N=160)</b>	38	1	7	114
<b>Aptima™ assay (N=301*)</b>				
<b>Asymptomatic (N=141*)</b>	5	1	4	131
<b>Symptomatic (N=160)</b>	42	1	3	114

446 \*2 specimens gave invalid result

447  
 448

449 **Table 3:** Qualitative results for saliva and NPs using the Aptima™ assay

450

		Saliva		
		Positive	Negative	$\Sigma$
NPs	Positive	47	7	54
	Negative	2	245	247
	$\Sigma$	49	252	301*

\*2 specimens gave invalid result

451

452

453

454 **Table 4:** Qualitative results for five-saliva pools and individual saliva using the LDT assay

455

		<b>Pooled saliva</b>		
		Positive	Negative	$\Sigma$
<b>Individual saliva</b>	Positive	40	4	44
	Negative	3	254	257
	$\Sigma$	43	258	301*

\*2 specimens gave invalid result

456

457 **Table 5:** Qualitative results for pools of five saliva specimens and individual saliva using the  
 458 Aptima™ assay

		Pooled saliva		
		Positive	Negative	$\Sigma$
<b>Individual saliva</b>	Positive	42	7	49
	Negative	0	252	252
	$\Sigma$	42	259	301*

\*2 specimens gave invalid result

459

460 **Figure legends**

461

462 **Figure 1:** IP2 (A) or IP4 Ct values (B) for paired NPs and saliva samples. Data are means +

463 SD. \*\*\*\* P  $\leq$  0.0001 (Student's paired t-test).

464

465 **Figure 2:** IP2 (A) or IP4 Ct values (B) for asymptomatic and symptomatic individuals. Data

466 are medians plus interquartile range (IQR). \* P  $\leq$  0.05 \*\* P  $\leq$  0.01 (Mann-Whitney U-test).

467

468 **Figure 3:** IP2 (A) or IP4 Ct values (B) for paired positive NPs and saliva individuals and NPs

469 positive only individuals. Data are medians and IQR. \*\*\*\* P  $\leq$  0.0001 \*\*\* P  $\leq$  0.001 (Mann-

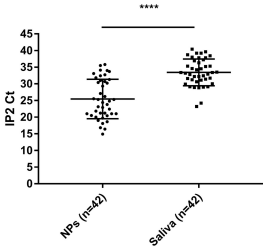
470 Whitney U-test).

471

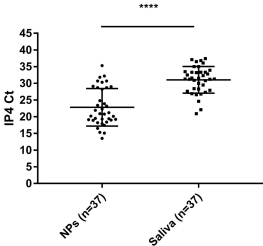
472



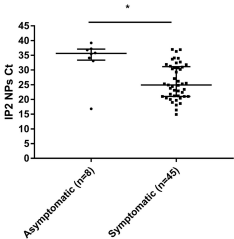
# 1.A



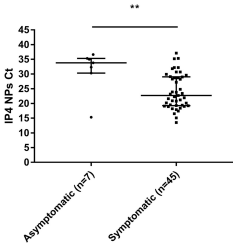
# 1.B



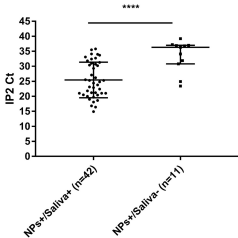
## 2.A



## 2.B



### 3.A



### 3.B

