Saliva sampling is an excellent option to increase the number of SARS CoV2 diagnostic tests in settings with supplies shortages

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Running title: A simpler and faster method to diagnose SARS CoV-2

Abstract

As part of any plan to lift or ease the confinement restrictions that are in place in many different countries, there is an urgent need to increase the capacity of laboratory testing for SARS CoV-2. Detection of the viral genome through RT-qPCR is the golden standard for this test, however, the high demand of the materials and reagents needed to sample individuals, purify the viral RNA, and perform the RT-qPCR test has resulted in a worldwide shortage of several of these supplies. Here, we show that saliva samples treated with a lysis buffer can serve as a suitable source for viral RNA detection, that is cheaper and can be as efficient as the classical protocol that involves column purification of the viral RNA. In addition, it skips the need for swab sampling, decreases the risk of the healthcare personnel involved in this process, and accelerates the diagnostic procedure.

INTRODUCTION

With the worldwide COVID-19 health emergency, there is an urgent need for rapid and reliable methods of diagnostic for SARS-CoV-2. The accepted golden standard for detection of this virus is the amplification of regions of the viral genome by RT-qPCR in nasopharyngeal and oropharyngeal swabs[1][2]. Unfortunately, given the enormous demand of the reagents needed to take the biological samples, and to purify the viral RNA, there have been shortages of many of the reagents needed for the diagnostic tests. Swabs, viral transport media, and kits for viral RNA extraction are amongst the most common consumables that have become scarce, compromising the number of tests that can be done in many parts of the world.

39 Recently, several reports have demonstrated the possibility of using saliva instead of oral
40 and nasal swabs to detect the genome of SARS-COV-2 [3][4][5]. Saliva collection also has many
41 collateral benefits, including self-collection, what decreases the risk of healthcare workers in
42 charge of taking the swabs, and does not require the use of PPE (personal protecting equipment)
43 that has also become a scarce item in this pandemia [6][7]. In addition, the methods to extract
44 the RNA from biological samples require the use of purification kits whose availability has also
45 become limited due to the heavy worldwide demand.

46 In this report, we compared the RT-qPCR results from 253 paired samples obtained from
47 saliva and swabs of ambulatory patients; the RNA in the swab samples was extracted using a
48 commercial RNA purification kit, and the saliva samples were directly mixed with a lysis buffer,
49 boiled, and used for the RT-qPCR protocol. We found a very good correlation of results between
50 both types of samples, and propose that this method, which simplifies the sampling of patients,
51 and accelerates the preparation of the RNA for the RT-qPCR test represents an excellent
52 alternative that will facilitate to sample and diagnose a larger number of persons at a reduced
53 cost.

54

55 **MATERIALS AND METHODS**

56 **Sample collection.** 253 paired samples from oropharyngeal and/or nasopharyngeal swabs, and
57 saliva were collected during a span of 30 days (from May 2nd to 31st) by healthcare workers from
58 the epidemiology department of the health ministry of the state of Morelos (Secretaría de Salud
59 Morelos, SSM). All but 3 samples, were from ambulatory patients, the 3 exceptions were
60 collected from hospitalized patients. After their collection, swabs were placed in 2.5 ml of viral

61 transport medium. Saliva were self-collected by patients that were asked to spit several occasions
62 into sterile urine cup containers until completing roughly 2-3 ml, both samples were maintained
63 at 4ºC and transported to the Institute of Biotechnology, UNAM for their analysis, within 24 hours
64 after sample collection.
65
66 **Nucleic acid extraction and SARS-CoV-2 detection by RT-qPCR.** Total RNA was extracted from
67 swab samples using the QIAamp viral RNA mini kit (QIAGEN) following the manufacturer´s
68 protocol, using 140 μl of viral transport medium from each swab and the RNA was eluted in 60
69 μl of elution buffer.
70 Saliva samples were treated with the Quick Extract TM DNA Extraction Solution (QE,
71 Lucigen) by mixing 50 μl of saliva with 50 μl of QE and heating for 5 minutes at 95ºC, the mixture
72 was then cooled on ice and kept at 4ºC until their use (within 1 hour of QE treatment). In saliva
73 samples that had high viscosity, 1 volume of sterile phosphate-buffered saline (PBS) was added
74 and mixed by repeated pippeting, the diluted saliva sample was the extracted as mentioned
75 above.

76 SARS-CoV-2 detection was performed using the Berlin protocol, using the reported oligos
77 and probes for viral gene E and for human RNase P [19]. The RT-qPCR reactions were performed
78 using the StarQ One-Step RT-qPCR (Genes 2 Life) kit, using 5 μl of the column extracted total RNA
79 in a 20 μl of reaction mix, or 2.5 μl of the QE treated saliva into 22.5 μl of reaction mix. Samples
80 were analyzed in an ABI Prism 7500 Sequence Detector System (Applied Biosystems) with the
81 following thermal protocol: 50ºC for 15 min, 95ºC for 2 min and then 45 cycles of 95ºC for 15 s
82 and 60ºC for 30 s. All samples with a Ct value equal or less than 38 were classified as positive.

83 **Determination of viral copy number.** To determine the viral copy number in a sample, a standard
84 curve was generated using a 10-fold serial dilution of an *in vitro* T7 RNA transcript that encodes
85 the sequence recognized by oligonucleotides and probe for gene E. Briefly, the logarithm of
86 concentration of each dilution was plotted against the Ct and the viral copy number from
87 unknown samples was determined by extrapolating the Ct value onto the corresponding
88 standard curve.

89 **Statistical analysis.** Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad
90 Software Inc.) as described in the results section.

91

92 **RESULTS**

93 **Viral titer determination in swabs and saliva.** To evaluate if saliva is a good source of viral RNA
94 for the RT-qPCR tests we determined the presence of the SARS-CoV-2 genome in paired saliva
95 and swab samples from 253 ambulatory patients. All patients had two or more symptoms related
96 to COVID-19. All patients (100%) had two or more symptoms related to COVID-19, 115 (45.4%)
97 were male and 137 (54.1%) female, with a median age of 41 (+/- 14.4) years (Table 1)[8][9].
98 Samples were taken from ambulatory patients in the respiratory triage of the Tlaltenango health
99 Center, in Cuernavaca, Morelos. The RT-qPCR Berlin protocol was used to detect SARS-CoV-2,
100 using only the primers and probe for gene E, since previous studies have shown a weak detection
101 of viral RNA when the RdRp gene is probed [10][11]. As an internal control of RNA content in the
102 samples, the RNase P gene was detected. Total RNA was purified from swabs using the QIAamp

103 viral RNA mini kit, the RNA in saliva was directly obtained using the QE lysis buffer (Lucigen) and
104 boiled for 5 minutes, as reported [12].

105 From the 253 samples analyzed, 139 (54.9 %) were negative for the presence of SARS-
106 CoV-2 both in swab and saliva samples, and 114 (45.1%) were positive for SARS-CoV-2 in at least
107 one type of sample. Out of the 114 positive samples, 60 (52.6%) were positive both in swab and
108 saliva, while 34 (29.8%) were positive only in saliva, and 20 (17.5%) were detected only in swabs
109 (Figure 1). According to these results, saliva samples allowed the detection of 82.4% of total
110 positive samples, while through swabs only 70.1% positives were detected.

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112 **Quantitation of viral RNA.** When the number of viral copies in the positive paired samples were
113 compared, a significant difference between the geometric mean of the viral copy number in saliva
114 and swab samples was detected, with saliva samples having a 1.2 $\log_{10}$ higher titer than that
115 observed in swabs ($p<0.005$, Figure 2A). This can be better appreciated when the viral copy
116 number in paired swabs and saliva from the same patient, is plotted and represented as
117 connecting lines (Figure 2 B); in 42 of the paired samples the number of viral copies was higher
118 in saliva samples than in swabs. Human RNase P was used as an internal control of sampling
119 quality; the comparison between the mean of Ct values obtained from swabs and saliva showed
120 a difference of at least 7.6 Ct's between both types of samples (Figure 2 C), indicating that there
121 is more cellular material in saliva, as reported in other studies[13].

122 Taken together these results suggest that that saliva is a good source of SARS-CoV-2, and
123 can be used for diagnostic tests using QE extraction buffer in place of the column-based method.

124 The higher virus titer as well as the higher level of RNase P detected in saliva as compared to
125 swabs, support this conclusion.
126
127 **DISCUSSION**
128 In this study we analyzed 253 paired samples from pharyngeal and/or nasopharyngeal
129 swabs and saliva. RNA purified from swabs using commercial column kits was compared with
130 saliva treated by direct lysis using QE buffer and surpassing the RNA extraction protocol. More
131 positive samples (82.4%) and a higher viral copy number (1.2 $\log_{10}$) were detected in saliva as
132 compared to the paired nasopharyngeal swab (70.1%) samples. In this regard, the number of
133 false negatives in swabs was almost twice (29.8%) as those in saliva samples (17%).
134 The failure of identification of SARS-CoV-2 in swab samples when the saliva samples were
135 positive for the virus could be due to bad swab sampling, what can be corroborated by the high
136 Ct values of RNase P detected in these samples (Figure 2 C), with the consequent low viral copy
137 number. This is a major concern, since for natural reasons the medical personnel in charge of
138 taking the samples frequently do not do it correctly for the risk associated with this process. Of
139 note, most (28/34) of the samples that were positive for saliva and negative for swabs
140 corresponded to swab samples that were taken only from the oropharinx (single swab). It has
141 been reported that nasopharyngeal swabs have a higher viral titer compared with oropharyngeal
142 swabs [2]; thus, this could contribute to the discrepancies observed. In addition, it has also been
143 previously reported that nasopharyngeal swabs have a lower viral titer than saliva samples [13],
144 what can also contribute to explain our findings. On the other hand, the false negatives in saliva

145 could be due to problems during the collection, transport and or storage of the sample before its
146 arrival to the laboratory.

147 SARS-CoV-2 has been detected in saliva at higher titers during the first days after the
148 onset of symptoms, with the viral titer declining over time. It is not clear how long after the
149 symptom onset the viral RNA can be detected in saliva, although some reports suggest a short
150 period of detection (~13 days) as compared with nasopharyngeal swabs (~19 days) [14].
151 However, other reports have recently demonstrated the detection of viral RNA in saliva for longer
152 periods of time (~20 days or longer) [3][15]. The patients included in this study were ambulatory,
153 and according to their clinical interview had between 1 and 7 days, (with a median of 4 days) of
154 the onset of symptoms. We did not find a significative difference between the onset of symptoms
155 and the results obtained from samples that were only positive in saliva versus those that were
156 only detected in swabs.

157 Direct lysis of nasopharyngeal or oropharyngeal swab samples in viral transport medium
158 using the QE buffer has been reported as a suitable method for direct qRT-PCR for SARS-COV-2
159 detection, with rates similar to methods based on column purification [16][12]. However, in our
160 experience we have found a great variability in the results obtained using the QE lysis protocol
161 when applied to swab samples, most likely due to variations in the material of the swabs used
162 and to variations in the preparation of the viral transport medium employed (data not shown).
163 In this regard, it has recently been reported that the composition of viral transport media can
164 affect the detection of viral RNA from SARS-CoV-2 and other viruses [17] and, due to the scarcity
165 of it, several laboratories have started to prepare their own transport media introducing an
166 additional confusion factor. A similar situation occurs with the swabs, since in view of the scarce

167 suitable materials, other materials are being employed, despite the fact that some materials are
168 known to inhibit the RT-PCR reactions [18].

169 The use of saliva samples offers the advantage that, in general, saliva has a constant
170 composition and no additives or transport media need to be used for their preservation if stored
171 in cold and analyzed up to 36 h after their collection. Our results indicate that a rapid processing
172 of saliva using direct lysis with QE buffer, and avoiding an RNA extraction protocol, offers an
173 excellent alternative to the current swab analysis that uses RNA column purification, since it is a
174 sensitive, fast and cheap method that can be used for massive screening, in particular in those
175 settings where common supplies needed for the classical methods are in shortage.

176

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179 invaluable help in collecting the samples, and to the personnel of the Laboratorio Estatal de Salud
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182

183 **FIGURE LEGENDS**

184 **Figure 1. Comparation of SARS-CoV-2 positive samples in swab and saliva**. Percent number(n)
185 of positive samples (+) and negative samples (-) detected in swabs and saliva as indicated.

186

187 **Figure 2. SARS-CoV-2 is detected at higher titer in total positive saliva samples.** A) Viral titer
188 (viral copies/ml) from all positive samples detected in swabs (n=94) and in saliva (n=80); B) Viral

189 titer (viral copies/ml) in positive swab and saliva samples (n=60) were represented by lines
190 connected between both samples. Data were compared by a Wilcoxon test ($p<0.005$); C) RT-PCR
191 cycle threshold Ct values for RNase P. Data were compared by Mann-Whitney test ($p< 0.005$ and
192 $p<0.0001$). Bars represent the geometric median and 95% CI.
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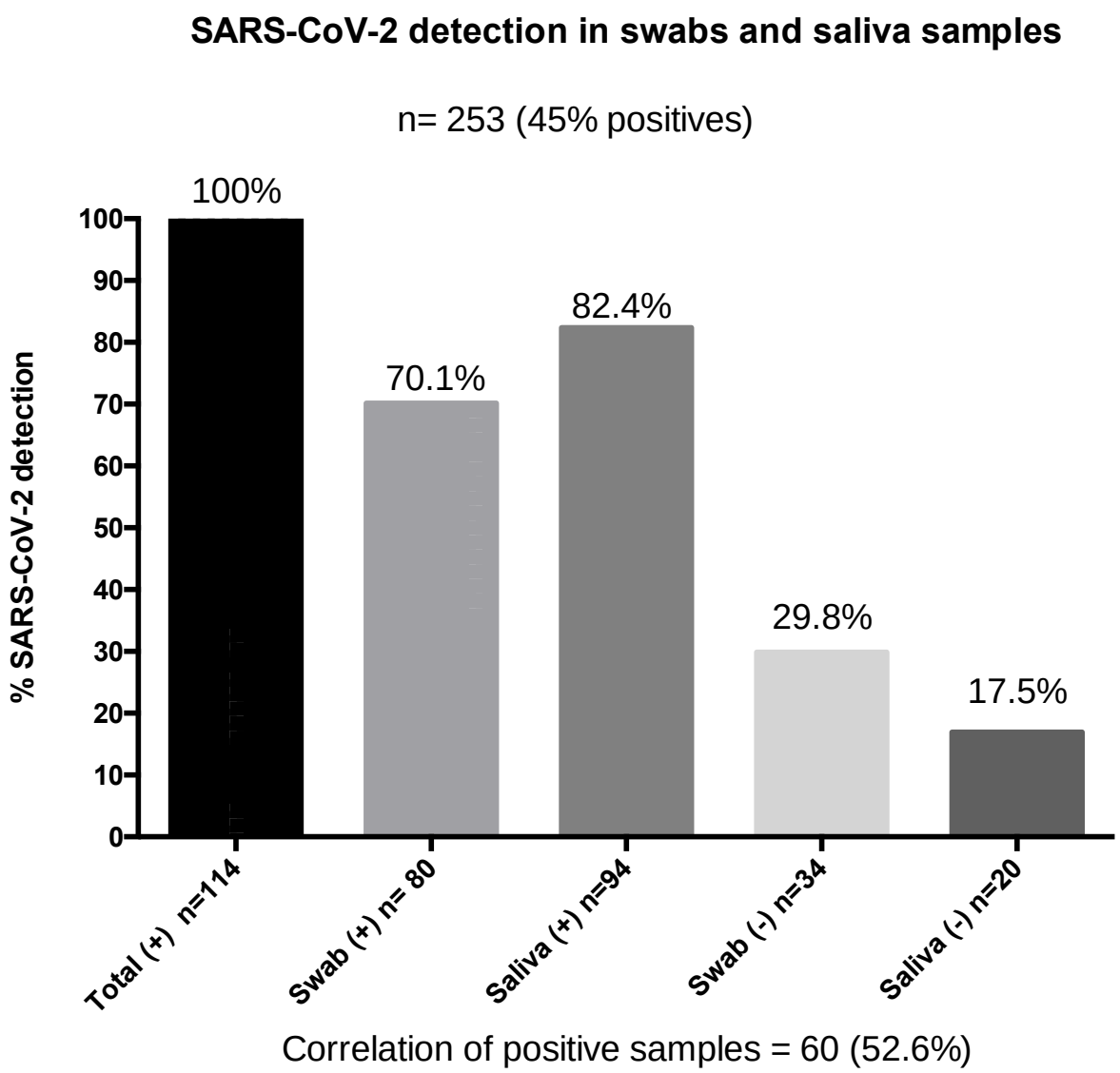
SARS-CoV-2 detection in swabs and saliva samples
n= 253 (45% positives)
% SARS-CoV-2 detection
100
90
80
70
60
50
40
30
20
10
0
100%
70.1%
82.4%
29.8%
17.5%
Total (+) n=114
Swab (+) n= 80
Saliva (+) n=94
Swab (-) n=34
Saliva (-) n=20
Correlation of positive samples = 60 (52.6%)

Figure 1

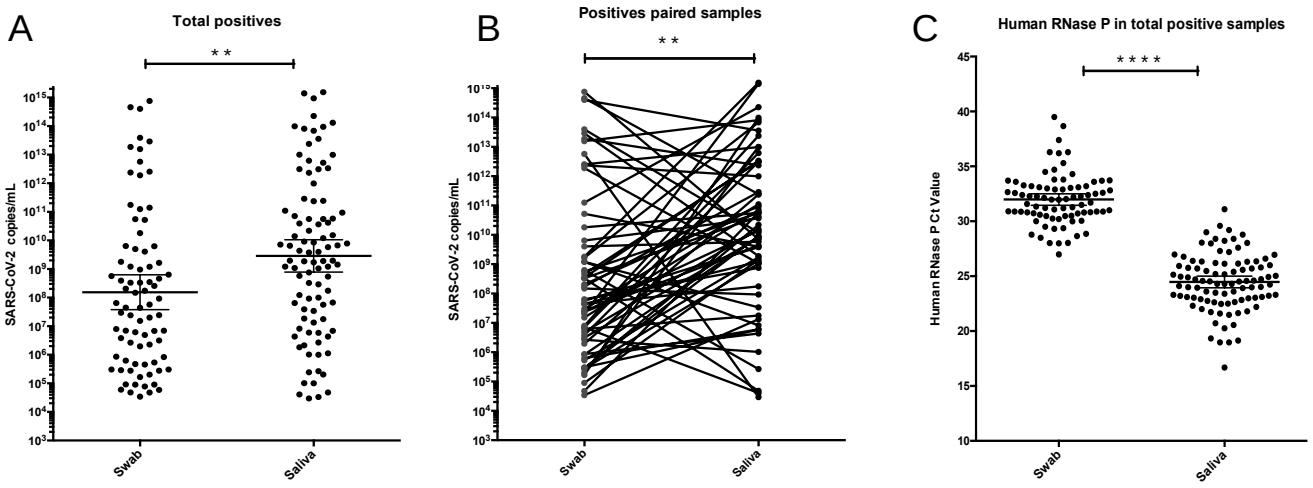
A
Total positives
* *
SARS-CoV-2 copies/mL
Swab
Saliva
B
Positives paired samples
* *
SARS-CoV-2 copies/mL
Swab
Saliva
C
Human RNase P in total positive samples
* * * *
Human RNase P Ct Value
Swab
Saliva

Figure 2

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TABLE 1. Patient data

	Total paired samples (253)*
Male	115 (45.4%)
Female	137 (54.1%)
Age mean	41 +/- 14.4 years
Hospitalized	3 (1.1%)
Ambulatory	229 (90.5%)

*Data from some patients were not obtained

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