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Usefulness of a Quantitative Olfactory Test for the Detection of COVID-19

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Abstract

Background: During the COVID-19 pandemic, olfactory dysfunction (anosmia or hyposmia) has been reported by many patients and recognized as a prevalent and early symptom of infection. This finding has been associated with viral-induced olfactory neuron dysfunction rather than the nasal congestion typically found in cold- or flu-like states. In the literature, the prevalence of anosmia varies from 15% to 85%, and the studies, in general, were based on the subjective evaluation of patients' self-reports of loss of smell (yes or no question). In the present study, we quantitatively evaluated olfactory dysfunction and the prevalence of fever in symptomatic patients suspected of having COVID-19 using a scratch-and-sniff olfactory test and infrared temperature testing with RT–PCR as the gold-standard comparator method to diagnose COVID–19 infection.

Methods: The forehead temperature of outpatients was checked with an infrared noncontact thermometer (temperature gun). After that, they received two olfactory smell identification test (SIT) cards (u-Smell-it™; CT, USA) that each had 5 scent windows and were asked to scratch with a pencil and sniff each of the 10 small circles containing the microencapsulated fragrances and mark the best option on a response card. Nasopharyngeal swabs were then collected for reverse transcriptase–polymerase chain reaction (RT–PCR) to determine whether the patients were positive or negative for COVID–19. We considered the number of hits (correct answers) ≤ 5 as positive for loss of smell (LOS) in the olfactory test; ≥ 6 hits were considered negative for LOS (i.e., normal olfactory function). All the data were analyzed using Excel and MATLAB software.

Results: One hundred sixty-five patients who were eligible for the olfactory test and nasopharyngeal swab collection RT–PCR were included. Five patients were excluded because of inconclusive PCR results (n=2) or missing data (n=3). A total of 160 patients completed all the protocols. The RT–PCR positivity rate for COVID-19 was 27.5% (n=44), and compared with RT–PCR-negative patients, RT–PCR-positive patients scored significantly worse on the olfactory test (5.5±3.5) (8.2±1.8, $p<0.001$). None of the PCR-positive patients presented with fever (≥37.8°C). In contrast, an olfactory SIT had a specificity of 94.8% (95% CI, 89.1–98.1), a sensitivity of 47.7% (95% CI, 32.7–63.3), an accuracy of 0.82% (95% CI, 0.75–0.87), a positive predictive value of 77.8% (95% CI, 59.6–88.8), a negative predictive value of 82.7% (85% CI, 78.7–86.7), and an odds ratio of 16.7.

Conclusion: Our results suggest that temperature monitoring failed to detect COVID-19 infection, while an olfactory test may be useful for identifying COVID-19 infection in symptomatic patients.

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Keywords: COVID-19, Olfactory Dysfunction, Diagnosis, RT-PCRIntroduction

Before the advent of the SARS-CoV-2 vaccine and large-scale immunization of the population worldwide, coronavirus disease 2019 (COVID-19) caused severe injury and death. It quickly became a pandemic, as the transmission of the virus often occurred before the onset of symptoms. To further complicate matters, the symptoms associated with COVID-19 were variable, and many could be mild and hard to identify objectively or even not present at all (asymptomatic). Thus, it was a challenge for science and medicine to stop rapid viral transmission and identify suspected cases of this wide range of symptoms. Among the clinical manifestations of COVID-19, olfactory dysfunction (OD), which consists of anosmia or hyposmia, was frequently reported by many patients, and studies based mainly on patient surveys have indicated that OD is a prevalent and early symptom of SARS-CoV-2 infection [1-4]. In contrast to nasal congestion typically found in viral upper respiratory tract infections, COVID-19-induced OD is associated with the presence of the virus in cells adjacent to olfactory neurons [5] and, by a mechanism that, until recently, has not been fully elucidated, causes alterations in the odor perception function of olfactory neurons [5]. The rapid onset of anosmia is highly suggestive of SARS-CoV-2 viral infection, and in many cases of COVID-19, OD and ageusia are the only presenting symptoms [3,4,6]. However, OD, especially hyposmia, may be unnoticed unless it is formally and objectively tested with a measurable olfactory test. There is a wide range for the prevalence of COVID-19-associated OD in the literature, from 15% to 85%, and most of the data are from subjective evaluations of patients' self-reports of loss of smell [1,7-9]. The use of an olfactory test to diagnose COVID-19 infection has been reported in different studies during the pandemic [7,9-13]. However, nearly all those studies only tested the OD in PCR-positive patients. Thus, the test's specificity and degree of association with the disease (full odds ratio) are unclear. Similarly, how quantitative olfactory tests compare to temperature testing, a quantifiable symptom commonly used for COVID-19 screening, is unclear.

Population testing is a crucial strategy for efficiently identifying and isolating suspected cases, mainly because of the high infectivity of COVID-19. The principal tools for diagnosing COVID-19 are reverse transcriptase-polymerase chain reaction (RT–PCR) and antigen tests. Although PCR is the gold standard with excellent sensitivity and specificity, it is costly. It requires sample collection by others, special handling, instrumentation, and analysis—often at distant locations—which can cause significant delays. These issues pose challenges for very wide-scale population testing and are largely confined to testing a subpopulation of suspected infected people. There is a global need for an alternative, affordable, reliable, albeit imperfect, test to be used on a large scale to identify infected individuals and block transmission more easily. Here, we compared a scratch-and-sniff-style smell identification test (SIT) that has five odorant windows on a single card as a potentially quick, inexpensive, and easy prescreen test for COVID-19 in symptomatic patients, with RT–PCR as the reference standard to investigate the feasibility of using a quantifiable olfactory test to help identify COVID–19 infection. Additionally, we examined the effectiveness of infrared forehead temperature screens in the same patient cohort.

Methods

The study was conducted at the Center for COVID-19 Diagnosis of the Federal University of Rio de Janeiro (UFRJ). A total of 165 individuals who presented with mild cold-like symptoms at our center were enrolled from June to August 2020. Nasopharyngeal swabs were obtained from each participant, and a diagnosis of COVID-19 was made via RT–PCR using the CDC protocol, with primers and probes for N1 and N2 targets. Clinical and demographic data were self-reported by the patients. The study was approved by the local ethics committee of Clementino Fraga Filho University Hospital (CAAE: 30161620.0.0000.5257). Written informed consent was obtained from all participants. The inclusion criterion was symptomatic outpatients older than 18 years who were scheduled to have their nasopharyngeal swabs collected for PCR at the UFRJ testing facility. Patients who had rhinorrhea or nasal congestion were excluded, and eligible volunteers whose forehead temperature was checked with an infrared noncontact thermometer (temperature gun) were excluded. After that, the volunteers received two u-Smell-it™ (Connecticut, USA) olfactory SIT cards (5 scents each) and were asked to scratch and sniff each of the ten areas containing the microencapsulated fragrances and mark the best choice of 5 options (4 scent choices and ‘no scent’) on a response card. In our protocol, three versions of the test were used. Cards #1414 and #1515 have the same scents; however, they are presented in different orders, and card #1313 has different scents from #1414 and #1515—supplemental Figures 1 and 2 present examples of card tests and response cards, respectively. For the first protocol, we used one card, #1313, in combination with another card, #1414 or #1515, to have 10 different smells presented to the patients. For the second protocol (reproducibility analysis), we used #1414 and #1515 cards to have 2 smells repeated on both cards. Supplemental Table 1 shows the scent options and the right response for all cards used in this study.

For tests with two olfactory cards (10 scents), the optimal cutoff to most accurately distinguish COVID-19-positive and COVID-19-negative patients, as determined by RT–PCR, was achieved using ≤5 correct responses (‘hits’) scored as ‘positive’ for loss of smell (LOS); ≥ 6 hits were

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Cutoff (<)	Sensitivity	95% CI	Cutoff (<)	Specificity	95% CI
1	0.16	0.06 – 0.30	1	0.98	0.94 – 0.99
2	0.2	0.10 – 0.35	2	0.98	0.94 – 0.99
3	0.3	0.17 – 0.45	3	0.97	0.93 – 0.99
4	0.3	0.17 – 0.45	4	0.97	0.93 – 0.99
5	0.34	0.20 – 0.50	5	0.96	0.90 – 0.98
6	0.48	0.32 – 0.63	6	0.95	0.89 – 0.98
7	0.5	0.35 – 0.65	7	0.9	0.83 – 0.94
8	0.59	0.43 – 0.73	8	0.78	0.69 – 0.85
9	0.75	0.60 = 0.87	9	0.55	0.46 – 0.65
10	0.93	0.81 – 0.99	10	0.2	0.14 – 0.29

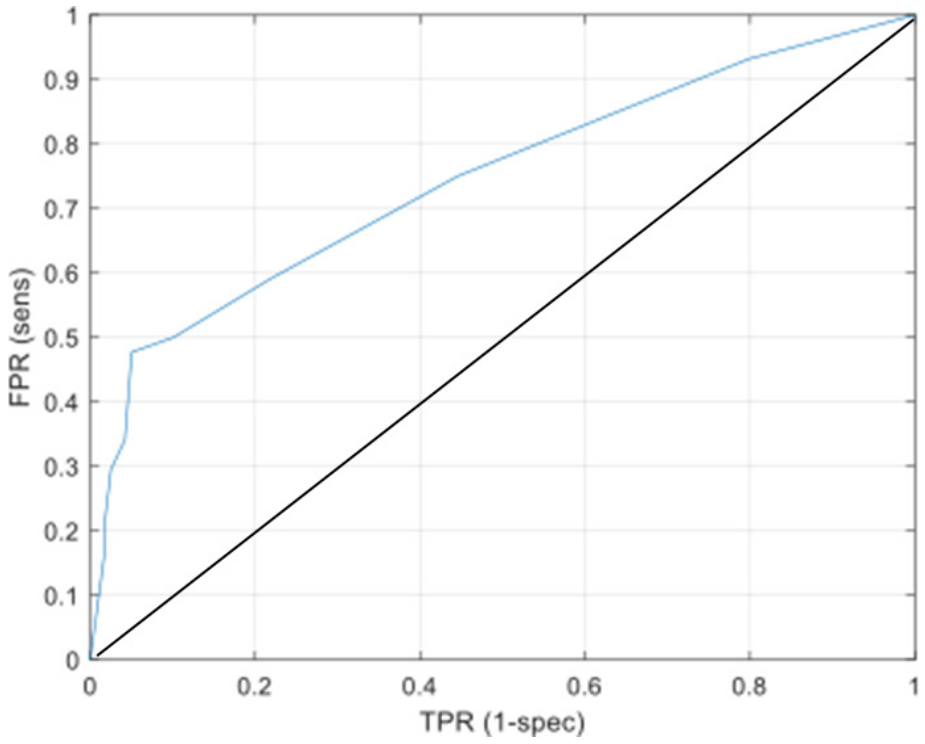

Figure 1: Sensitivity, specificity, and ROC curve for u-Smell-it™ using two cards (10-scent).

Cutoff (<)	Sensitivity	95% CI	Cutoff (<)	Specificity	95% CI
1	0.19	0.18 – 0.23	1	0.98	0.98 – 0.98
2	0.31	0.27 – 0.36	2	0.97	0.96 – 0.98
3	0.41	0.36 – 0.45	3	0.94	0.92 – 0.96
4	0.55	0.50 – 0.59	4	0.81	0.77 – 0.83
5	0.82	0.77 – 0.86	5	0.44	0.39 – 0.48

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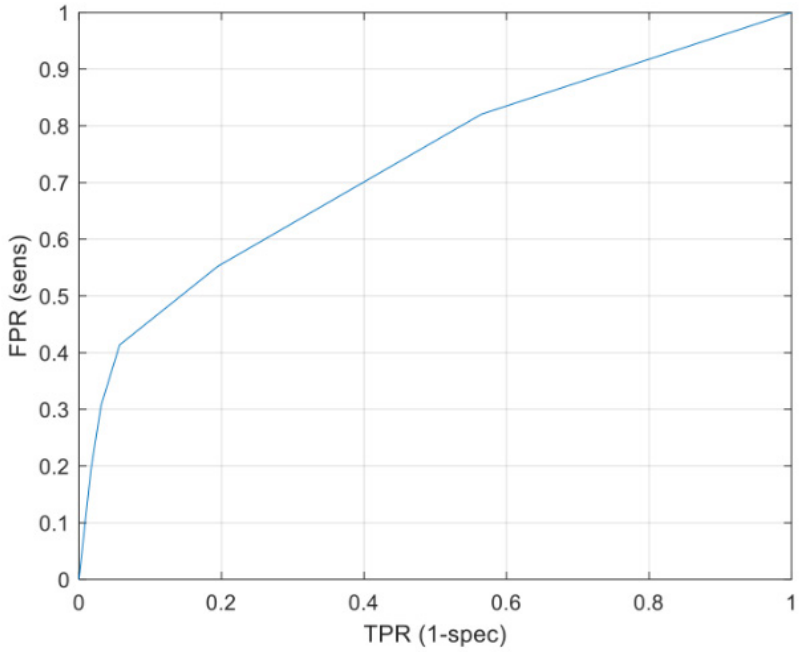

Figure 2: Sensitivity, specificity and ROC curve for the use of u-Smell-it™ as a single card (5-scent).

Table 1: Demographic data.

		All patients	RT–PCR+ patients	RT–PCR– patients
		(n=160)	(n=44)	(n=116)
Age		38.3±13	39.5±12.4	35.5±13.2
Gender	M (n, %)	54 (33.8)	20 (45.4)	34 (29.3)
	F (n, %)	106 (66.2)	24 (54.6)	82 (70.7)
Forehead temp (^{0}C)		36.4±0.2	36.4±0.2	36.34±0.2
Self-report LOS (n, %)		60 (37.5)	28 (63.6)	32 (27.5)
u-Smell-it mean score		7.5±2.7	5.5±3.5*	8.2±1.8

LOS, loss of smell.

considered negative for LOS. Figure 1 shows the sensitivity and specificity for different cutoffs and the ROC curve for the test using two cards. To evaluate whether the use of a single olfactory card (5 scents) presented similar results to the use of two olfactory cards (10 scents), we used a bootstrap statistical procedure (see below) to simulate the use of a single card and determine the statistical results. Figure 2 shows the sensitivity and specificity for different cutoffs after the bootstrap statistical procedure and the ROC curve. With the goal of testing for a positive association between the u-Smell-it™ olfactory test and RT–PCR-based COVID-19 diagnosis, two binary variables were considered, one for the tested scheme and another for the gold/reference standard diagnosis. We constructed contingency tables and calculated the sensitivity, specificity, and positive and negative predictive values. We also applied the chi-square Pearson test [14,15] to demonstrate that the proposed method does not produce an independent outcome compared to the gold standard diagnosis. Bootstrap [16] is a statistical procedure that creates many simulated samples by resampling the dataset. The parameters are estimated by averaging the results of all runs. Here, we randomly sorted 5 out of 10 odorants for each patient and calculated the parameters after passing through the complete dataset. This procedure was repeated 10,000 times for the whole dataset, generating 10,000 values for each parameter. New random draws were performed for each patient in all

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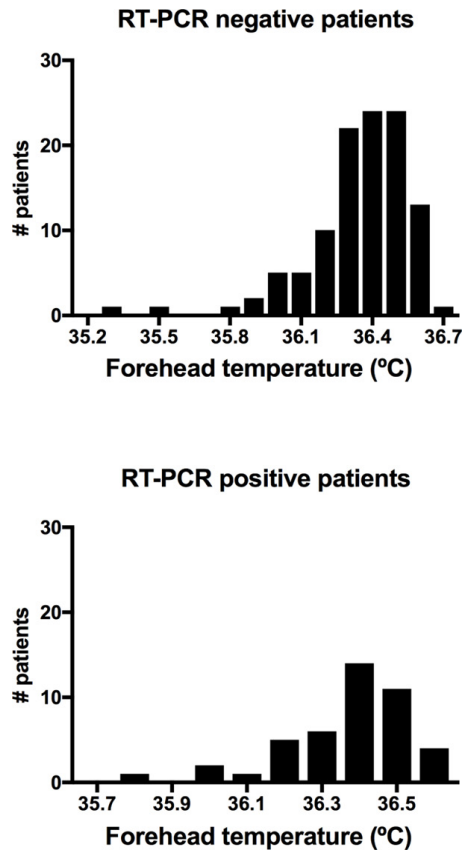

Figure 3: Frequency distribution of the forehead temperature of patients according to RT–PCR results.

Table 2: u-Smell-it™ olfactory test (two cards) vs RT–PCR.

		RT–PCR	
		Positive	Negative
u-smell-it™	Positive	21	6
	Negative	23	110

Table 3: Statistical findings of the u-Smell-it™ olfactory test (two cards) vs RT–PCR

	Mean	95% IC
Sensitivity	47.7%	32.5 – 63.3%
Specificity	94.8%	89.1 – 98.1%
PPV	77.8%	59.6 – 88.8%
NPV	82.7%	78.7 – 86.7%
LR+	9.2	4.0 – 21/3
LR-	0.6	0.4 – 0.7
OR	16.7	6.1 – 46.1
Accuracy	82%	75 – 87%
p value	<0.0001	
Chi stat	41	

PPV, predictive positive value; NPV, negative predictive value; LP+, positive likelihood ratio; LR-, negative likelihood ratio; OR, odds ratio.

Table 4: Self-reports of loss of smell vs RT–PCR.

	PCR	
Self-report LOS	Positive	Negative
Positive	28	32
Negative	16	84

LOS, loss of smell.

Table 5: Statistical findings when comparing self-reports of LOS to PCR.

	Mean	95% IC
Sensitivity	63.6%	47.8 – 77.3%
Specificity	72.4%	37.1 – 55.3%
PPV	46.0%	59.6 – 88.8%
NPV	84.3%	78.2 – 89.0%
LR+	2.3	1.6 – 3.3
LR-	0.5	0.3 – 0.8
OR	4.6	2.2 – 9.6
Accuracy	70%	62 – 77%
p value	<0.0001	
Chi stat	41	

PPV, predictive positive value; NPV, negative predictive value; LP+, positive likelihood ratio; LR-, negative likelihood ratio; OR, odds ratio.

the runs. In the end, the average of these 10,000 runs was used to estimate the parameters and confidence intervals, as calculated through the 2.5th and 97.5th percentiles. For reproducibility, a two-branch experiment was run with five odorants in each for the same group of individuals to evaluate differences between branch responses. The Wilcoxon signed-rank test was used for post hoc analyses. All the data were statistically analyzed using MATLAB software (R2019b) and confirmed with MedCalc® software.

Results

One hundred sixty-five patients were eligible for the olfactory test and nasopharyngeal swab collection for PCR. Five patients were excluded because of inconclusive PCR results (n=2) or missing data (n=3). A total of 160 patients completed the full protocol. The PCR positivity rate for COVID-19 was 27.5% (n=44). The demographic data are shown in Table 1. The forehead temperature check did not reveal any fever (0/165), which was defined as a temperature of 37.8°C or above. The frequency distribution of the temperatures is shown in Figure 3. The contingency table and the statistical analysis of the olfactory test performance using two u-Smell-it™ cards (10 scents) compared with the RT–PCR results for SARS-CoV-2 are shown in Tables 2 and 3, respectively. Olfactory testing showed a specificity of 94.8% (95% CI, 89.1–98.1), sensitivity of 47.7% (95% CI, 32.7–63.3), positive predictive value of 77.8% (95% CI,

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59.6–88.8), negative predictive value of 82.7% (85% CI, 78.7–86.7), accuracy of 82% (95% CI, 75–87), and odds ratio of 16.7. The contingency table and the statistical analysis of self-reports of LOS performance compared with RT–PCR are shown in Tables 4 and 5, respectively. Although the sensitivity and PPV of self-reports of the LOS test were greater than those of the u-Smell-it™ quantitative olfactory test, the specificity, NPV, and accuracy of self-reports were lower. Interestingly, using an RT–PCR test as a reference standard, the odds ratio for olfactory dysfunction, as determined by the u-Smell-it™ olfactory quantitative test, was 3.6-fold greater than the odds ratio of self-reported LOS (4.6).

To evaluate the reproducibility of the quantitative olfactory test, another group of 66 patients was tested with two u-Smell-it™ cards containing the same five scents but arranged in a different order. Forty-one out of 66 tested individuals produced the same number of correct hits in both branches; 23 had a divergence of just one scent, and two patients mismatched two or more scents. Wilcoxon signed rank analysis produced a p value= 0.58, indicating that there is no evidence that the 'smells' were perceived as diverse in the two branches of tests. Subjects did not report any side effects or complaints about test participation. Most patients took approximately 90 sec or less to complete the test with two olfactory cards (10 scents).

Discussion

The present study showed that a large fraction of SARS-CoV-2 PCR-positive patients had olfactory dysfunction, and this symptom, when detected by an olfactory test, was highly specific (95%). Our results demonstrated that the odds ratio of the u-Smell-it™ test for detecting COVID-19 infection was 3.6-fold greater than that of self-reports, suggesting that a quantitative test outperforms self-surveys. These findings were observed in the aggregate and after using a cutoff score of three on a 6-point scale (LOS = score 0,1,2 out of 5 maximum). The results indicate that a simple 5- or 10-window olfactory smell identification test can specifically differentiate, with only approximately 5% false positives, people infected by SARS-CoV-2 with 82% accuracy. Interestingly, the statistical performance metrics of the five-window test were similar to those of the 10-window test, which is consistent with the relatively intense loss of smell in our study population. Dramatically, in contrast to olfactory tests, temperature checking failed to detect any cases of COVID-19 infection. This study underscores this weakness and likely the limited usefulness of using temperature tests and infrared camera monitoring to identify persons infected with COVID-19. Our results showed that none of the 44 patients who tested positive for SARS-CoV-2 had a fever (typically a temperature greater than 38°C). Notably, fever is a nonspecific symptom of viral infection, and because of that, the usefulness of temperature screening for identifying suspected cases has been called into question [17–20]. Nevertheless, body temperature checks are routinely applied as the primary screening test to identify individuals with fever at the entrance to many public places, such as schools, airports, hospitals, etc.

Notably, the literature shows that only approximately 20% of people who have COVID-19 have a fever, which generally occurs very early in the course of the infection and has a short duration (under three days) [21]. The body temperature of our SARS-CoV-2-positive patients was not significantly different from that of SARS-CoV-2-negative patients (Table 1 and Figure 1), and one possible explanation is that the outpatients observed in our study population may have arrived after transient fever. It also shows the poor performance of temperature-based screens and further questions their usefulness as a screening test. Our study tested two different symptoms of COVID-19 infection (OD and temperature) and provided strong evidence that an olfactory test would significantly outperform a temperature test. Another key finding of the study was that an olfactory test significantly outperformed self-reported LOS. Specifically, for patients who were positive for COVID-19 according to RT–PCR, the odds ratio for self-reports was only 4.6, while for patients who were positive according to a real olfactory test, it was 16.7. This result suggested that patients with OD who were diagnosed using olfactory tests were more strongly associated (3.6×) with a positive test for COVID-19 than with self-reported LOS. This finding is consistent with other reports in which an objective test outperformed nonobjective and nonquantitative surveys [22]. Patients who did not have a LOS data included those with both anosmia and hyposmia.

Our results showed that the olfactory test specificity for detecting OD in patients infected with COVID-19 was very high (~95%), which was consistent with other reports [23], including the CDC [24], indicating that "a new loss of taste or smell" was the single best indicator symptom of COVID-19, as indicated by the odds ratio. Although OD is acknowledged by many research teams and listed on the WHO website as a finding consistent with SARS-CoV-2 infection, little attention has been given to whether OD is a better indicator of COVID-19 than other symptoms. Considering this symptom's high specificity, the OD should be much better suited for rapid screening than the ubiquitous temperature test. The journal STAT News proposed that, for screening suspicious cases of COVID-19 infection, a smell test would be a better option than temperature tests [25]. The sensitivity of the test with u-Smell-it™ (ranging from 48% in the 10-scent test to 55% in the 5-scent test) was lower than expected, considering other reports that showed OD detection via smell testing with a sensitivity of 76% (51–91%)[23]. Given that no patients had fever in our population and that the average duration of loss of smell was reported to be approximately 7 to 8 days [11], one possible interpretation is that some patients may

have arrived after recovering from LOS. Consistent with this possibility, no patients had an elevated temperature above baseline. Second, for self-reported LOS, the sensitivity was greater; however, the specificity was very poor. Considering that the average duration of LOS is only approximately one week (note that a subset of patients (~10%) can have a longer-term loss) and that RT–PCR positivity may persist for three weeks or more, it is conceivable that, in our study, some patients were tested for OD after recovery; this is consistent with the finding indicating that they had a recent LOS on the questionnaire but showing standard performance in an olfactory test. However, properly addressing this issue would require longitudinal testing or other mechanisms, such as serology, to accurately identify disease staging.

The study's limitations include that we recruited only outpatients who came to the clinic for a diagnostic test at variable points of the disease, which, as mentioned above, we have not ascertained, and we did not investigate asymptomatic patients. Moreover, our cohort did not include children, pregnant individuals, or elderly individuals, so our results may not be directly generalizable to these groups of patients. It was anticipated that a short 5- or 10-odorant smell identification test for patients with minor hyposmia may be missed compared to a 40-odorant UP-SIT test [11,26].

In conclusion, our study demonstrated that a positive SARS-CoV-2 RT–PCR result was strongly associated with olfactory dysfunction, which was 3.6-fold greater in patients who were tested with a short olfactory test than in those who were detected via self-reports. The results of the present study suggest that quick olfactory tests may be useful for detecting COVID-19 infection in symptomatic patients.

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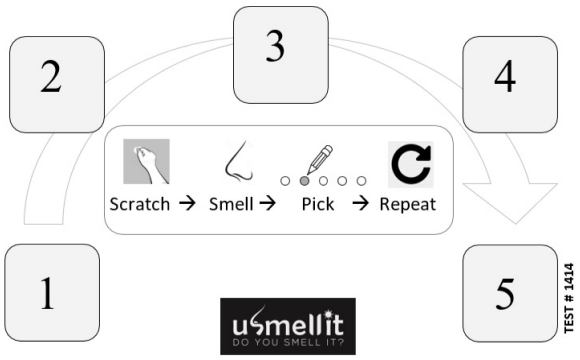
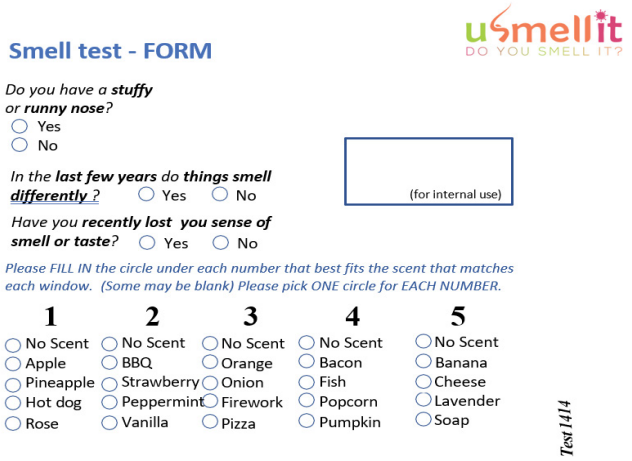
SUPPLEMENTARY FILES



Supplemental Figure 1: Example of a scratch-and-sniff (u-Smell-it™) olfactory test.



Supplemental Figure 2: Example of a response card to the olfactory test.

Supplemental Table 1: Scent options and the right response for all cards used in this study.

C (card 1414)	NO SCENT	NO SCENT	NO SCENT	NO SCENT	NO SCENT
	apple pineapple	barbecue strawberry	orange onion	bacon fish	banana cheese
	hot dog	pepper mint	fireworks	pumpkin	lavender
	rose	vanilla	pizza	popcorn	soap
D (card 1515)	NO SCENT	NO SCENT	NO SCENT	NO SCENT	NO SCENT
	popcorn fish	lavender cheese	fireworks onion	pepper mint strawberry	apple pineapple
	bacon	soap	orange	barbecue	hot dog
	pumpkin	banana	pizza	vanilla	rose
B (card 1313)	NO SCENT	NO SCENT	NO SCENT	NO SCENT	NO SCENT
	leather	bacon	banana	burnt rubber	apple
	garlic	blue cheese	cinnamon	coffee	coconut
	pizza peach	chocolate mango	popcorn smoke	grape lime	pine smoke
Note: real scents are underlined. Card 1414 and 1515 are the same; just changed order Card 1313 has different scents					

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