The 8-Odorant Barcelona Olfactory Test (BOT-8): Validation of a New Test in the Spanish Population During the COVID-19 Pandemic

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Abstract

Background: Most smell tests are difficult to implement in daily clinical practice owing to their long duration. The aim of the present study was to develop and validate a short, easy-to-perform, and reusable smell test to be implemented during the COVID-19 pandemic.

Methods: The study population comprised 120 healthy adults and 195 patients with self-reported olfactory dysfunction (OD). The 8-Odorant Barcelona Olfactory Test (BOT-8) was used for detection, memory/recognition, and forced-choice identification. In addition, a rose threshold test was performed, and a visual analog scale was applied. The Smell Diskettes Olfaction Test (SDOT) was used for correlation in healthy volunteers, and the University of Pennsylvania Smell Identification Test (UPSIT) was used for patients with OD to establish cut-offs for anosmia and hyposmia. In order to take account of the COVID-19 pandemic, disposable cotton swabs with odorants were compared with the original test.

Results: In healthy persons, the mean (SD) BOT-8 score was 100% for detection, 94.5% (1.07) for memory/recognition, and 89.6% (0.86) for identification. In patients with OD, the equivalent values were 86% (32.8), 73.2% (37.9), and 77.1% (34.2), respectively. BOT-8 demonstrated good test-retest reliability, with agreement of 96.7% and a quadratic k of 0.84 (P<.001). A strong correlation was observed between BOT-8 and SDOT (r=0.67, P<.001) and UPSIT (r=0.86, P<.001). Agreement was excellent for disposable cotton swabs, with a k of 0.79 compared with the original test. The cut-off point for anosmia was ≤3 (area under the curve, 0.83; sensitivity, 0.673; specificity, 0.993).

Conclusions: BOT-8 offers an efficient and fast method for assessment of smell threshold, detection, memory, and identification in daily clinical practice. Disposable cotton swabs with odorants were compared with the original test.

Key words: Olfaction. Smell test. Loss of smell. Anosmia. COVID-19.

Resumen

Introducción: Las olfatometrías son difíciles de implementar en la práctica clínica diaria por su larga duración. El objetivo del presente estudio fue desarrollar y validar una prueba simple, fácil y reutilizable para ser utilizada durante la pandemia de COVID-19.

Métodos: Se incluyeron 120 voluntarios sanos ≥18 años y 195 pacientes con disfunción olfatoria (DO) autoreportada. Se utilizó el Barcelona Olfactory Test (BOT-8) con 8 odorantes para la detección, memoria/reconocimiento e identificación. Además, se hizo una prueba de umbral de rosa (alcohol feniletílico) de 6 diluciones, escala visual analógica (EVA). Se compararon los resultados con una prueba validada Smell Diskettes Olfaction Test (SDOT), para definir puntos de corte de hiposmia y anosmia se comparó en pacientes con DO con UPSIT™. Considerando la pandemia de COVID-19, se compararon hisopos de algodón desechables con los odorantes respecto a la prueba original.

Resultados: BOT-8 se tarda entre 3 y 7 minutos en realizar. En población sana, la media de detección fue del 100%, memoria 94.5% (DE=0.86) e identificación 89.6% (DE=0.86). En pacientes con DO fue de 86% (DE=32.8), 73.2% (DE=37.9) y 77.1% (DE=34.2), respectivamente. BOT-8 demostró buena fiabilidad test-retest con 96.7% de concordancia observada y una kappa cuadrática de 0.84 (p<0,001). Presentó una fuerte correlación con SDOT (r=0.673, p<0,001) en población sana y con UPSITTM en pacientes con DO (r=0.86, p<0,001). Los hisopos de algodón desechables mostraron una excelente concordancia (kappa de 0.79) en comparación con la prueba original. El punto de corte para anosmia fue ≤3 (AUC=0.83, Se=0.673, Sp=0.993) y de hiposmia ≤6 (AUC=0.451, Se=0.088, Sp=0.814).
Introduction

Olfaction is the earliest and most primal sense [1,2]. The ability to identify and discriminate between smells reflects the health of the sinonasal cavity, cognitive state, and higher cortical centers [3].

Olfactory dysfunction (OD) is common in many conditions, including sinonasal diseases [4], postinfectious disorders [2], traumatic brain injuries [5,6], and neurodegenerative disorders [7], among others [8,9]. Olfactory function also plays an important role in daily living, eg, enjoyment of food, ability to detect spoilage, detection of safety hazards, socialization, and overall quality of life [10].

Methods for subjective measurement of OD are an important component of diagnosis and monitoring of treatment. Nowadays, one of the most common problems in olfactory tests is the time they take to perform and their difficult applicability in daily practice [11].

The scientific literature shows how the COVID-19 pandemic has raised awareness of OD in patients with SARS-CoV-2 infection [12]. Consequently, there is a clear need to adapt current olfactory tests to ensure that they can be applied safely by medical personnel and patients through single-use and/or self-administered tests [13]. This approach raises considerably the cost of examinations, creating the need for more cost-effective alternatives, such as disposable cotton swabs.

Aims and Objectives

The aim of the present study was to develop and validate a simple, easy-to-perform, and reusable smell test to be implemented over a short period (5 to 10 minutes). In addition, the test had to be adaptable and viable and comply with the necessary safety measures during the COVID-19 pandemic.

Patients and Methods

Participants

Two groups were recruited at Hospital Clinic, Barcelona, Spain. The first included healthy adult volunteers aged ≥18 years with no subjective loss of smell who were equally distributed by sex and in age groups of 10-year intervals. We excluded patients with upper respiratory tract infection in the previous 2 weeks, known psychiatric or neurocognitive impairment, head trauma, or sinonasal OD. We also excluded pregnant women and patients who had undergone nasal surgery.

We assessed a second group of participants with self-reported OD (age, ≥18 years). The inclusion criterion was loss of smell (visual analog scale [VAS], ≥30 mm).

All patients provided their signed informed consent to permit use of their data for scientific purposes. The study was approved by the Ethics Committee of our institution (HCB-2015-0076).

Testing Procedure

All the volunteers were tested individually in a well-ventilated room with controlled humidity and temperature (21-23°C). They were tested simultaneously in both nostrils, first with the 8-Odorant Barcelona Olfactory Test (BOT-8) to assess smell detection, recognition/memory, and forced-choice identification. The smell threshold was assessed using rose (phenylethyl alcohol). The total time taken to perform the test was measured (Figure 1A). To compare the results of our smell test with a previously validated and standardized smell identification test, all healthy volunteers were also tested using...
the Smell Diskettes Olfaction test (SDOT) [14], and all cases with self-reported OD were compared using the University of Pennsylvania Smell Identification Test (UPSIT) to define cut-off points for hyposmia and anosmia.

A smell VAS (with 0 mm representing no loss and 100 mm maximum loss) was administered and nasal endoscopy performed to evaluate septal deviation and turbinate hypertrophy and rule out the presence of nasal polyps in healthy controls.

A subgroup of 30 healthy adults were tested in 2 separate sessions with a 2-week interval to evaluate the test-retest reliability of BOT-8.

Considering the current situation of the SARS-CoV-2 outbreak and the safety limitations with respect to multiuse smell tests, a subgroup of 20 healthy adults were tested using disposable cotton swabs with odorants to evaluate agreement between the swab and the routine smell test (BOT-8) in order to favor single-use material during the COVID-19 pandemic, as recommended in guidelines [15] (Figure 1, B and C).

**Barcelona Olfactory Test (BOT-8)**

The BOT-8 is a supraliminal orthonasal subjective olfactometry test. The different odors were presented in random order using semi-solid-state odorants contained in glass jars and placed about 3 cm below the nostrils for 3-5 seconds, with a latency of 30 seconds between each smell. The 8 odorants (banana, chocolate, lemon, rose, coffee, onion, mint, and vinegar [Table 1]) were selected after analysis by an expert consultant based on international guidelines and the previously validated Barcelona Smell Test 24 (BAST-24).

Participants were asked to answer Yes or No to the following questions: (1) Can you smell anything? (detection); (2) Do you remember having smelt it before? (memory/recognition); and (3) Which of these 4 odorants is correct? (forced-choice identification).

The score was calculated independently for detection, memory, and identification as an absolute value and percentage, with 8/8 (100%) being the maximum score.

Smell is assessed based on detection, identification, and threshold. In this test, as in the BAST-24, the recognition/memory item was added, since we consider that smell has a cultural component and requires prior exposure to the odorant. Therefore, when asking patients if they have ever smelled it, we first know if they have been previously exposed to that odorant. It also tells us about the patient's olfactory memory.

### Rose (Phenylethyl Alcohol) Threshold Test

Six geometric dilutions were presented in glass jars placed 3 cm below the nostrils. The lowest dilution was presented first (1/1000) and progressively increased (1/500, 1/100, 1/50, 1/10, and 1/1 corresponding 15% from the pure essence) until the patient could detect the smell. The scale was reversed to lower concentrations when the odor was correctly identified in 2 successive tests, or towards higher concentrations when the odor was not recognized. The threshold was defined as the mean of the last 2 scale reversals [16].

### Smell Diskettes Olfaction Test

Our objective was to validate our test in a healthy population against a screening test capable of identifying normality from abnormality. To do so, we used the Smell Diskettes Olfaction test (SDOT) test.

SDOT is composed of 8 odorants (coffee, vanilla, peach, smoke, orange, rose, chocolate, and vinegar), which are applied using reusable diskettes as odor applicators. These floppy disks are made of polyester and measure 5 × 6 cm and can be opened to release odors and closed after testing.

The test is based on a triple-forced multiple choice (0 to 8 correct answers). Normal scores are defined as 6.2 (1.0) for the age group 18-50 years and 6.0 (0.9) for the age group 51-50 years.

### University of Pennsylvania Smell Identification Test

We used the University of Pennsylvania Smell Identification Test (UPSIT) test to be able to determine our cut-off points for hyposmia and anosmia in the affected population.

UPSIT is a self-administered olfactory identification test comprising 40 items (available in Spanish). It provides an absolute indication of loss of smell (anosmia; mild, moderate, or severe hyposmia), as well as a relative indication based on percentiles related to age and sex, with a test-retest r of 0.94.

In order to compare it with our test, which comprises 8 odorants, we reduced the UPSIT classification to 3 categories: anosmia, hyposmia (including mild, moderate, and severe categories), and normosmia.

### Statistical Analysis

Frequency and mean (SD) were calculated for the demographic and clinical characteristics of the participants. We performed an independent-sample t test to compare means between the sexes, and a Pearson correlation analysis was used to analyze the correlation between smell outcomes and age.

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**Table 1. Odorants Selected for the BOT-8 Identification Test With Their Chemical Compounds and Descriptors Used for the Forced-Choice Task**

<table>
<thead>
<tr>
<th>No.</th>
<th>Odorant</th>
<th>Chemical compound</th>
<th>From pure essence, %</th>
<th>Descriptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Banana</td>
<td>Isoamyl acetate</td>
<td>15</td>
<td>Vanilla, sausage, chicken, Tangerine,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>pineapple, soap</td>
</tr>
<tr>
<td>2</td>
<td>Chocolate</td>
<td>Pyrazines</td>
<td>5</td>
<td>Cheese, popcorn, fish</td>
</tr>
<tr>
<td>3</td>
<td>Lemon</td>
<td>Citral</td>
<td>15</td>
<td>Apple, honey, cookies</td>
</tr>
<tr>
<td>4</td>
<td>Rose</td>
<td>Phenethyl alcohol</td>
<td>15</td>
<td>Coconut, mustard, cherry</td>
</tr>
<tr>
<td>5</td>
<td>Coffee</td>
<td>Furans, pyrazines</td>
<td>5</td>
<td>Cinnamon, strawberry, ham</td>
</tr>
<tr>
<td>6</td>
<td>Onion</td>
<td>Dipropyl disulfide</td>
<td>10</td>
<td>Tomato, peach, gasoline</td>
</tr>
<tr>
<td>7</td>
<td>Mint</td>
<td>Menthol</td>
<td>15</td>
<td>Smoke, ammonia, orange</td>
</tr>
<tr>
<td>8</td>
<td>Vinegar</td>
<td>Acetic acid</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: BOT-8, 8-Odorant Barcelona Olfactory Test.
Agreement between BOT-8 and the cotton swab was determined using the weighted $k$ statistic, as described by Cohen [17]. The maximum $k$ statistic is 1.00, which indicates perfect agreement, with 0 indicating no agreement. We assessed the weighted $k$ statistic for strength of agreement using the guidelines of Fleiss et al [18]. Poor agreement was <0.40, good agreement was 0.40 to 0.75, and excellent agreement was ≥0.76.

The Pearson correlation was calculated between the BOT-8 items and the VAS, UPSIT, and SDOT scores. (“Poor” is less than 0.3, “fair” is 0.3 to 0.5, “moderately strong” is 0.6 to 0.8, and “very strong” is >0.8) [19].

The Cronbach $\alpha$ coefficient was calculated to determine the reliability of BOT-8 (the Cronbach $\alpha$ coefficient should have a minimum value of 0.7 for preliminary research) [20]. The test-retest reliability was assessed using the Cohen weighted $k$ coefficient for ordinal scales.

The performance of BOT-8 was assessed based on sensitivity, specificity, and positive and negative predictive values. The cut-off point for anosmia was set at ≤18 for UPSIT.

Data were managed and the statistical analysis was performed using IBM SPSS Statistics for Windows, Version 21 (IBM Corp., Armonk, NY, USA) with the $\alpha$ level set at 0.05.

Table 2. Demographics and Clinical Characteristics of the Controls (Healthy Volunteers) and Cases (Self-Reported Olfactory Dysfunction)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (Mean (SD))</th>
<th>Cases (Mean (SD))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD) age, y</td>
<td>47.7 (18.3)</td>
<td>51.0 (16.8)</td>
</tr>
<tr>
<td>Female sex, No. (%)</td>
<td>57 (47.5)</td>
<td>107 (54.9)</td>
</tr>
<tr>
<td>Smoking history, No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>– Current/Past</td>
<td>41 (34.2)</td>
<td>32 (16.6)</td>
</tr>
<tr>
<td>– Never</td>
<td>79 (65.8)</td>
<td>161 (83.4)</td>
</tr>
<tr>
<td>Septal deviation, No. (%)</td>
<td>83 (69.2)</td>
<td>64 (34.0)</td>
</tr>
<tr>
<td>Inferior turbinate</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hypertrophy, No. (%)</td>
<td>21 (17.5)</td>
<td>47 (25.0)</td>
</tr>
</tbody>
</table>

![Figure 2. B-Odorant Barcelona Olfactory Test. Smell detection, memory/recognition, and identification by sex (females vs males) and by age group.](image-url)
The Pearson correlation between smell detection and age was significant for memory/recognition and identification: \( r = -0.18 \) (95%CI, –0.31 to 0.04; \( P = .013 \)) and \( r = -0.19 \) (95%CI, –0.32 to –0.05, \( P = .008 \)), respectively. No significant correlation was found for detection: \( r = -0.07 \) (95%CI, –0.22 to 0.06, \( P = .28 \)).

**Reliability (Test-Retest)**

The Cronbach α coefficient was 0.837. BOT-8 test-retest reliability demonstrated excellent agreement with a weighted \( \kappa \) statistic of 0.84 and 96.7% observed agreement (95%CI, 0.67-0.99; \( P < .001 \)) (Figure 3).

**Agreement Between BOT-8 and Single-use Cotton Swabs**

The quadratic \( \kappa \) correlation between disposable swabs and BOT-8 identification was assessed, yielding 98.75% observed agreement and \( \kappa = 0.79 \) (95%CI, 0.78-0.81).

**VAS Score**

In healthy volunteers, the mean VAS score for smell loss was 11.4 (13.6) in women and 17.4 (15.5) in men. The Pearson correlation between the BOT-8 total score for identification and memory/recognition and the VAS was poor (\( r = -0.086 \) [95%CI, –0.32 to 0.05, \( P = .352 \)] and \( r = -0.115 \) [95%CI, –0.84 to –0.73], \( P < .001 \); memory/recognition, –0.79 [95%CI, –0.84 to –0.73], \( P < .001 \); and identification –0.86 [95%CI, –0.89 to –0.82], \( P < .001 \)).

**Correlation Between BOT-8 and Olfactory Tests**

In healthy volunteers, the mean SDOT score was 7.0 (0.1) and the BOT-8 identification score was 7.17 (0.9) (Table 3). The Pearson correlation between the BOT-8 identification score and SDOT was strong (\( r = 0.673 \), \( P < .001 \)).

In patients with OD, the mean UPSIT score was 22.7 (9.3) and the BOT-8 identification score was 6.2 (2.7) (Table 4). The Pearson correlation coefficient between BOT-8 identification and UPSIT was 0.86 (95%CI, 0.82-0.89) (\( P < .001 \)).

**Contingency Table for Anosmia and Receiver Operator Characteristic Curve**

In OD patients, the UPSIT score was categorized as normal or abnormal following the ≤18 cut point for anosmia. Using this value, a receiver operating characteristic curve was
plotted (Figure 4). The area under the curve (AUC) was 0.833 (95%CI, 0.762-0.904). Acceptable sensitivity and excellent specificity for anosmia was defined as ≤3 in BOT-8 (sensitivity, 0.673 [95%CI, 0.546-0.801]; specificity, 0.993 [95%CI, 0.979-1.000]). The positive likelihood ratio and the negative likelihood ratio were 0.972 (95%CI, 0.919-1.000) and 0.893 (95%CI, 0.845-0.941), respectively (Table 5).

The COVID-19 pandemic has highlighted the importance of validating the test safely for patients and health professionals. Application of cotton swabs with the odorant yielded excellent agreement, thus showing the test to be safe. We encourage application of disposable swabs and single-use tests, as recommended in guidelines [8]. Using this approach as an alternative when evaluating patients is less expensive than the single-use tests and safer in viral pandemic situations.

A recent meta-analysis by Wang et al [22] concluded that females outperformed males among young adults aged 18 to 50 years [23]. The OLFACAT survey revealed a similar result for all age groups in the Catalan population [15]. In our cohort, healthy females scored slightly better, with better olfactory outcomes than males. No differences in sex were found in the case of patients with OD.

We found higher identification scores in younger participants than in older ones. However, this tendency did not reach statistical significance in healthy volunteers (P=.06); in our opinion, the tendency would have been significant in a larger sample. We recorded higher identification and memory/recognition scores in younger patients (P=.013 and P=.008, respectively).

Discussion

In the present study, we developed and validated a supraliminal orthonasal olfactory test for adults. The advantages of the BOT-8 smell test are the short time needed for application (around 3 to 6 minutes) and the fact that it is easy-to-perform and reusable. Recently, the pediatric Barcelona Olfactory Test-6 (pBOT-6), which includes an identification and threshold test and was validated for Spanish children, demonstrated high sensitivity and specificity for detecting hyposmia [21].

We recorded a Cronbach α coefficient of 0.837, which indicates good internal consistency and excellent agreement in test-retest reliability. Moreover, the correlation coefficient was good when compared with the SDOT test (r=0.67) and very good when compared with UPSIT (r=0.86) in patients with OD. The BOT-8 identification score of ≤3 demonstrated acceptable sensitivity and excellent specificity for anosmia (0.673 and 0.993, respectively).

The COVID-19 pandemic has highlighted the importance of validating the test safely for patients and health professionals. Application of cotton swabs with the odorant yielded excellent agreement, thus showing the test to be safe. We encourage application of disposable swabs and single-use tests, as recommended in guidelines [8]. Using this approach as an alternative when evaluating patients is less expensive than the single-use tests and safer in viral pandemic situations.

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We found a poor correlation between the VAS and BOT-8 scores in healthy volunteers. Similar results were obtained by Zou et al [24], who reported that subjective ratings of olfactory function are overshadowed by many different aspects, such as motivation to seek counseling for loss of smell or coping with this situation. In contrast, in patients with OD, the VAS score correlated very well with scores for detection, identification, and memory/recognition in BOT-8.

The low reliability of self-reported olfactory function (eg, based on a VAS) and the numerous difficulties associated with the actual tests in daily practice (time-consuming, expensive, and mostly not reusable) reveal the need for more cost-effective OD tests. The BAST-24 is a validated Spanish cross-cultural smell test [25], whose main disadvantage is the long completion time (20–40 minutes). The UPSIT [26] is a validated single-use test applied throughout the world, with the considerable advantage that it can be self-administered, although it also has a high cost per test. Sniffin’ sticks [27], which is also applied worldwide, is reusable and affordable, although it is not disposable or single-use. In addition, transmission of COVID-19 by fomites continues to be a controversial issue [28].

The cost of commercially available tests for assessment of OD precludes their widespread use in clinical practice [29]. The need for more cost-effective tools in this area makes BOT-8 a good option owing to its fast application, reasonable cost per kit, and the fact that it can be reused with or without disposable cotton swabs over its 1-year lifetime.

Our study is subject to a series of limitations. We applied the test exclusively in Spanish patients and did not compare the performance of the test on patients from other cultures. Therefore, we recommend that the test be validated in other cultures. Furthermore, the test we chose for validation in the healthy population is not among the most widely used in the current literature and discriminates only between abnormality and normality. Therefore, in order to define cut-off points for hyposmia and anosmia, we used the UPSIT, which does have cut-off points for hyposmia and anosmia and has been validated in the Spanish population [26].

## Conclusions

BOT-8 is an efficient, fast, and easy-to-perform method for assessing smell threshold, detection, recognition/memory, and identification in adults in clinical practice. The test correlated very well with validated smell tests and showed high agreement in test-retest reliability. In the setting of the COVID-19 pandemic, disposable cotton swabs showed high agreement with the original test and proved to be a safe and economic alternative to self-administered single-use smell tests. Therefore, we propose our test as a useful screening tool for OD in Spanish patients and one that can be used not only by ENT specialists, but also by allergists, chest physicians, internists, and general practitioners.

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## Conflicts of Interest

- Isam Alobid: Consultant for Roche, Novartis, GlaxoSmithKline, Viatris, Menarini, MSD, and Salvat.
- Joaquim Mullol: member of national or international advisory boards. JM has received speaker fees and funding for clinical trials and research projects from ALK, AstraZeneca, Genentech, GlaxoSmithKline, Glenmark, Menarini, Mitsubishi-Tanabe, MSD, Mylan-MEDA Pharma, Novartis, Regeneron Pharmaceuticals, SANOFI-Genzyme, UCB Pharma, and Uriach Group.

The remaining authors declare that they have no conflicts of interest.

## References


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