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Screening of olfactory function using odourized markers*

J. Vodička[1,2], A. Pellant[1,2], V. Chrobok[1]

[1] Department of Otorhinolaryngology & Head and Neck Surgery, Regional Hospital Pardubice, Pardubice, Czech Republic

[2] Institute of Health Studies, University of Pardubice, Pardubice, Czech Republic

SUMMARY

Background: *The goal of our study was to create a psychophysical test for the screening of olfactory function on the basis of commercially available odourized markers (OM). There are six coloured markers in one package filled with different odourants at suprathreshold levels. In order to identify the best approach, we investigated five different variations of the technique.*

Materials and methods: *Olfaction was investigated in 189 subjects. Healthy participants as well as patients suffering from olfactory disorders were tested. Initially subjects were tested by one of five methods using OM. Finally, the "Sniffin' Sticks" test (butanol odour threshold, odour identification) was performed.*

Results: *Correlation of the OM screening test and the "Sniffin' Sticks" ranged from 0.49 to 0.93 indicating that variations of the technique strongly influence the results of testing. The best technique for evaluating olfactory function included spontaneous naming of odours and odour identification from a list of four distractors. The sensitivity of this method was sufficient to determine anosmia.*

Conclusions: *The odourized markers screening test can be used to screen for anosmia in the general population. However, the precise quantification of olfactory function is not possible, because of the relatively small amount of odours.*

key worlds: odourized markers, Sniffin' Sticks, olfaction, screening test

INTRODUCTION

The sense of smell and its disorders are frequently neglected by physicians. Although many tests of subjective olfactometry have been introduced, it is a particular challenge to find a valid olfactory test that is both affordable and readily available.

Impaired olfactory function decreases the quality of life and can lead to life threatening and hazardous events (gas-poisoning, cooking-related incidents or ingestion of spoiled food) [(1)]. Early diagnosis of olfactory disturbances can initiate preventive precautions. For example, olfactory screening could help identify individuals at high risk of being exposed to toxic substances, because their inability to smell poses a hazard. This is particularly important for employees in certain jobs, such as workers in chemical factories, who can be exposed to high doses of volatile, toxic chemicals.

Assessment of the olfactory function prior to nasal surgery is important from the medico-legal point of view [(2)].

Nasal obstruction and change of smell is the most common complaint of patients suffering from sinonasal disease [(3)]. Olfactory dysfunction can be a symptom of neurological disorders. Olfactory tests can help to distinguish Alzheimer's disease from other types of dementia [(4)] and the diagnosis of Parkinson's syndrome can be supported by olfactometry as well [(5)]. Thus, inexpensive olfactory tests would be useful.

To identify the most reasonably priced standardized test to assess olfactory function, we decided to use odourized markers (Figure 1), which are originally designed for use by children. We chose a package that included 6 coloured and odourized markers (Centropen®a.s., Art. 2589/6 Perfumes, Dacice, Czech Republic), each with a different colour, and each having a unique odour. The purpose of this study was to establish a simple, short and valid technique for olfactory screening on the basis of OM.

MATERIALS AND METHODS

The study was performed according to the Declaration of Helsinki (Summerset West amendment) on guidelines for biomedical research involving human subjects. It was approved by

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the Ethics Committee of the Regional Hospital Pardubice. All subjects provided written consent after they were thoroughly acquainted with all details of the investigation.

Figure 1. Commercially available odourized markers.

Participants

Olfactory functions were assessed in 189 subjects. Mean age of participants was 47.4 years, standard deviation 16.8 (age range 16 – 83 years); 115 men and 74 women took a part in our study. Healthy people as well as patients suffering from olfactory disorders were tested. We included patients and staff of the Department of Otorhinolaryngology & Head and Neck Surgery of the Regional Hospital Pardubice. Participants assessed their sense of smell as normal in 105 cases, decreased in 43 cases, completely impaired in 36 cases, and altered in 5 cases. A total of 74 people did not suffer from any disease (15 of whom were smokers) that could influence olfaction and were verified as to having normal olfactory function. A total of 68 patients suffered from sinonasal disease. Head trauma, upper respiratory tract infection, and idiopathic etiology were presented in 12, 14, and 4 patients, respectively. Seventeen patients suffered from diseases that could influence olfaction (psychiatric diseases, neurodegenerative diseases, tracheotomized patients and people exposed to toxic substances).

Study design

Initially subjects were tested with one of five techniques using odourized markers (OM). Finally, the "Sniffin´ Sticks" test (butanol odour threshold, odour identification) was performed. In addition, the subjects' history was taken and nasal endoscopy was performed. For evaluation of each technique, we included both normosmic subjects and subjects with olfactory loss as ascertained by means of the "Sniffin' Sticks".

Olfactory testing

Subjects were first tested with the OM set, then with "Sniffin' Sticks". Testing was performed in a quiet room with adequate ventilation.

The OM screening test includes 6 coloured and odourized pens. The black pen smells like liquorice, the yellow pen like lemon, brown like cinnamon, blue like raspberry, green like apple, and the red pen smells like strawberry. The exact chemical composition of the cartridges of pens is the trade secret of the producer. Pens are filled with water-soluble pigments and aromas. The OM are designed for children from 3 years of age and are non-toxic. The product matches the requirements of European Norm (EN-71) used for safety of toys. The producer guarantees the quality for at least of 2 years. The odours of the markers are of the same intensity when properly used, meaning that the top of the pen has to be covered properly after each use. There is standard filling of the markers in the factory with a quality control process in their manufacturing. A random control of the final product is performed.

In order to test odour identification, forced choice technique was used. The five techniques employed differed in the following aspects: (1) presented distractors were different, (2) repeated naming of odours was added to one of the techniques, (3) spontaneous naming was used in two techniques. When olfaction was tested with the OM set, subjects were blindfolded to prevent visual identification in four techniques. Colours of markers were uncovered only in Experiment B meaning that in this experiment subjects knew the colour of the odourized markers.

Description of techniques used

Based on results of three Preliminary experiments, we created 2 new techniques of testing olfaction and decided to validate them in a larger number of subjects. In the following, we will first describe the 3 Preliminary experiments preceding Experiments A and B.

Preliminary experiment 1: The black marker was presented first for 4 s and subjects were asked to select the appropriate descriptor from a list of four distractors (in this case, it was "liquorice", "raspberry", "paprika" and "hospital"). Then, they were asked to select another probable distractor. Thus, subjects selected two descriptors and labeled them as more appropriate or less appropriate. If subjects selected the correct answer (e.g., "liquorice") they received 2 points. If subjects had a "near miss" (e.g., "hospital"), they received another point (see Table 1 for descriptors and scoring).

Table 1. List of distractors of Preliminary experiment 1. Correct answers are shown in boldface (2 points), "near misses" are italic (1 point).

liquorice	raspberry	paprika	*hospital*
glue	**lemon**	leather	*perfume*
paprika	*clove*	**cinnamon**	coffee
mushroom	garlic	*strawberry*	**raspberry**
deodorant	**apple**	spice	meat
mint	**strawberry**	tomato	thinner

A maximum of 3 points for one odour presentation was possible. The same method was used for the yellow, brown, blue, and green markers. Because of the very artificial odour of the red marker, only one appropriate descriptor had to be selected. For the correct answer subjects received 2 points. The intention was to minimize the influence of the poorly identifiable odour of the red marker. The minimum and maximum score from this test was 0 and 17 points respectively.

Preliminary experiment 2: The principle of the second technique remained the same. We changed some of the incorrect descriptors (e.g. "paprika" to "cigarette" for the brown marker) and the correct descriptors as well ("strawberry" to "soap" for the red marker). We predicted greater differentiation between hyposmia and normosmia based on described changes than the results indicated.
The selection of two distractors (proper and "near miss") in Preliminary experiment 1 and 2 was not always understood by the subjects and the explanation of the technique prolonged the testing time. Therefore we decided to change the technique of testing.

Preliminary experiment 3: Spontaneous naming was added to the third technique. People were asked to name the odours first. Each of the markers had to be described by a different name. Subjects got one point for naming each of the odours differently (in total 6 points). Then the list of 4 distractors for every colour was presented. Some of the incorrect distractors were changed. The subjects had to choose only one descriptor. When the identification of the odour was correct, participants received 2 points, if subjects had a "near miss", they gained only 1 point. The purpose of this preliminary experiment was to eliminate the artificial character of the odours by enabling subjects to name the odours based on their own experiences. Descriptors were changed based on experiences with previous techniques.

Experiment A: Repetitive naming of odours was added to this definitive technique. People were asked to identify the odour from a list of 4 distractors for black, consecutively for yellow and brown marker. The list of distractors was the same as in Preliminary experiment 3. Subjects could choose only one distractor. If the answer was right, they gained 2 points for each odour. If the answer was "near miss", they gained 1 point. The same markers were presented again (black, yellow, brown), but in a different, randomized sequence. Subjects were asked to identify and name the odours again based on their previous identification from list of distractors. They gained another point, if the answer was right. The same procedure was done with the blue, green and red colours. For identifying red colour from 4 distractors subjects gained only one point to minimize the influence of this very artificial odour. The minimum and maximum score was 0 and 17 points respectively. The purpose of this technique was to increase the amount of smell stimulus by repetitive presentation of the odours.

Experiment B: This definitive technique was based on spontaneous naming and odour identification from the list of four distractors. People were asked to name the odours first. Each of the markers had to be described by different names. Subjects scored one point for naming each of the odours differently. If they could not name the odour or gave the same name to the odours, they scored 0 point. Then subjects had to choose one correct answer from the list of four distractors. The list was changed radically in order to respect the colours of the markers (Table 2). For example, when the yellow marker was presented, distractors of yellow colours were offered ("banana", "lemon", "apple" and "pineapple"). Subjects gained one point for the correct identification. The minimum and maximum score was 0 and 12 points respectively.

Table 2. List of distractors (Experiment B). The correct answers are boldfaced.

liquorice	pepper	paprika	currant
banana	**lemon**	apple	pineapple
chocolate	tea	**cinnamon**	coffee
grapefruit	strawberry	orange	**raspberry**
paprika	**apple**	kiwi	banana
orange	mandarin	**strawberry**	currant

"Sniffin' Sticks"
The comparison of olfactory function was performed by "Sniffin' Sticks" (threshold and odour identification), which is based on pen-like odour dispensing devices [6]. Odour discrimination, which is a part of "Sniffin' Sticks" test, was left out due to time constraints. Odour thresholds were determined using n-butanol as the odourant. For odour identification, 16 odourants were presented to each subject. In order to identify the odourant, a list of 4 descriptors was presented. The exact technique of testing is described by Hummel et al. [6].
When subjects scored less than 9 of 16 points in odour identification and were not able to detect n-butanol in its highest concentration in threshold testing, the subject was determined to be functionally anosmic.

Nasal endoscopy
After olfactory tests were completed, nasal endoscopy (Karl Storz, Hopkins Optic, 30°, 2.7mm, 11cm) was performed to assess possible pathology in the nasal cavity and nasopharynx.

Statistical analysis
General participant data from each experiment are presented in Table 3. Data were further investigated using SPSS 12.0 for Windows®. Correlation coefficients of threshold, identification, and OM test were calculated. Correlations were performed between scores from the various tests and age of each subject (Table 4).
For Experiment A and B t-tests were used to compare data of threshold, identification and OM screening test between

patients with sinonasal and posttraumatic olfactory loss, and for nasal endoscopicy. ROC (Receiver/Operator Characteristics) analysis was done to evaluate sensitivity and specificity for both techniques (Table 5).

Table 3. Number of subjects, number of patients with functional anosmia stated by "Sniffin' Sticks", mean age and standard deviation, and sex of participants in each experiment.

experiments	experiments	number of anosmics	mean age of participants ± standard deviation	sex of participants male/ female
Preliminary 1	22	4	46.5 ± 15.9	9 / 13
Preliminary 2	17	3	51.8 ± 17.7	15 / 2
Preliminary 3	25	7	45.1 ± 18.1	17 / 8
Experiment A	54	12	46.8 ± 16.2	37 / 17
Experiment B	71	7	47.9 ± 16.6	37 / 34
total	189	33	47.4 ± 16.8	115 / 74

Table 4. Correlation coefficients of all techniques with "Sniffin' Sticks" (butanol odour threshold and odour identification) and age.

experiments	threshold	identification	age
Preliminary 1	0.746	0.927	-0.433
Preliminary 2	0.546	0.493	-0.196
Preliminary 3	0.870	0.856	0.001
Experiment A	0.736	0.648	-0.527
Experiment B	0.707	0.747	-0.158
age	-0.247	-0.270	1.000

Table 5. Sensitivity and specificity of Experiment A and B of OM test for functional anosmia stated by "Sniffin' Sticks".

Eperiment A			Eperiment B		
cutoff value	sensitivity	specificity	cutoff value	sensitivity	specificity
2	0.08	1.00	0	0.14	1.00
3	0.17	1.00	1	0.43	1.00
4	0.42	0.98	2	0.43	0.98
5	0.58	0.98	3	0.57	0.97
7	0.67	0.98	4	0.86	0.95
8	0.83	0.93	5	1.00	0.94
9	0.83	0.83	6	1.00	0.91
10	0.92	0.74	7	1.00	0.81
11	0.92	0.62	8	1.00	0.67
12	0.92	0.50	9	1.00	0.56
13	1.00	0.33	10	1.00	0.36
14	1.00	0.24	11	1.00	0.19
15	1.00	0.10	12	1.00	0.00
16	1.00	0.00	-	-	-

RESULTS

Analyses of results from Preliminary experiment 1, 3, Experiment A and B indicated significant correlation between results from these techniques and testing with the "Sniffin' Sticks" test battery ($p<0.01$). Correlation coefficients of all techniques are presented in Table 4. A negative correlation was found in all techniques, except Preliminary experiment 3, between scores from the OM tests and age, which was in line with the results from the "Sniffin' Sticks".

Preliminary experiments were used to create the final method of olfactometry using odourized markers. Therefore, we present only the final results of Experiment A and B.

Subjects suffering from sinonasal diseases achieved lower scores in both Experiments when compared to healthy subjects ($p<0.01$). The difference between scores from patients with posttraumatic olfactory loss and healthy controls was also significant for OM test in both Experiments ($p<0.01$).

Nasal endoscopy revealed polyps in 15 subjects of both Experiments. Results of olfactory tests (Experiments, butanol odour threshold and odour identification) of subjects with polyps were significantly decreased in Experiment B ($p<0.05$), but only with regard to threshold testing in Experiment A (p values of identification and OM test were 0.07 and 0.32, respectively). Sensitivity and specificity of the technique of Experiment A for the diagnosis of anosmia were 100% and 33% and for Experiment B 100% and 94%, respectively (ROC analysis) (Table 5). These results suggest the second technique as acceptable for the screening of olfactory function.

DISCUSSION

Many olfactory tests have been established. "Sniffin' Sticks" and UPSIT (University of Pennsylvania Smell Identification Test) are among the most commonly used tests in Germany and the USA, respectively. Both tests provide valid information on olfactory function. "Sniffin' Sticks" test enables in its extended version threshold testing using n-butanol. UPSIT is based only on suprathreshold testing using 40 odours. It is not possible to perform a threshold measurement with any number of suprathreshold stimuli. To satisfy the need for a shorter screening test, both methods have their own shorter variations. Hummel et al. described a method using 12 common odours to distinguish severe olfactory dysfunction from normal olfactory function [7]. The test has advantages in terms of costs, because it can be used repeatedly for approximately 1 year. Shorter and faster variations of UPSIT are also available. Doty et al. described the development of the Cross-Cultural Smell Identification Test (CC-SIT) [8], which includes 12 odours and can be administered in less than 5 minutes. Advantages of this test are self-administration technique and incorporation of "multicultural" odourants. In addition, 3-item smell identification test Q-SIT (Quick Smell Identification Test) has been reported to screen for patients with anosmia. On the other hand, the specificity of the Q-SIT for anosmia was only 40%. There are other reports on the screening test of olfaction [9-10].

The present study investigated the application of odourized markers in the screening of olfactory function. Only suprathreshold testing is possible using OM. We decided to compare our test with the "Sniffin' Sticks". This decision was based on 4 reasons: (1) the technique of pen-like odour dispensing devices is similar, (2) odours in daily life of Germans and Czechs do not differ much, (3) threshold and suprathreshold testing is possible using "Sniffin´ Sticks" test and (4) the

test is reliable in detecting anosmia [6].

The final technique (Experiment B) of testing smell ability using OM was developed on the basis of several preliminary experiments. Negative correlation of OM screening test scores with age and the significant correlation to "Sniffin´ Sticks" test results (threshold and odour identification) validated its usefulness for olfactory screening. Additionally, this conclusion is supported by significantly decreased scores on an OM screening test in subjects suffering from sinonasal and posttraumatic olfactory loss. Good sensitivity (100%) and satisfactory specificity (94%) were achieved with regard to detecting anosmia.

Advantages of the present test are: low cost (approximately € 1,- per set), the possibility of repetitive use of one test, and its availability in regular shops. Among the major disadvantages of the OM screening test are: small numbers of tested items, the colours of the markers, and the artificial character of odours. Despite these facts, we conclude that OM screening test (presented in Experiment B) can be used for orientation assessment of olfactory function and as a screening method of anosmia in the general population.

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Jan Vodicka
Department of Otorhinolaryngology
& Head and Neck Surgery
Regional Hospital Pardubice
Kyjevská 44
532 03 Pardubice
Czech Republic

Tel: +42-04-6601 5311
E-mail: jan_vodicka@centrum.cz