

# Sniffin' Sticks Screening 12 test: Presentation of odours on filter paper improves the recognition rate\*

Kerstin Wirkner<sup>1,2</sup>, Andreas Hinz<sup>3</sup>, Markus Loeffler<sup>1,2</sup>, Christoph Engel<sup>1,2</sup>

<sup>1</sup> LIFE - Leipzig Research Centre for Civilization Diseases, University of Leipzig, Leipzig, Germany

<sup>2</sup> Institute for Medical Informatics, Statistics, and Epidemiology, University of Leipzig, Leipzig, Germany

<sup>3</sup> Department of Medical Psychology and Medical Sociology, University of Leipzig, Leipzig, Germany

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## To the Editor:

Monitoring of olfactory function and diagnosis of olfactory disorders using the pen-based "Sniffin' Sticks test" is problematic during the SARS-CoV-2 pandemic due to hygienic concerns. The aim of this study was to find out whether the results of olfactory testing obtained by presenting odours on a single-use filter paper is identical to performing the test by presenting the odour pens according to the manufacturer's manual.

A detailed explanation of the methods is provided in the supplementary data. Briefly, olfactory function in study participants was assessed using the "Sniffin' Sticks screening 12 test". The odours were presented (a) according to the standard procedure indicated by the manufacturer by sniffing the pen ('pen') and (b) after painting odour lines on a strip of filter paper and sniffing the paper strip ('paper'). Depending on the number of correctly identified odours, the participants were classified as "normosmic", "hyposmic" and "anosmic".

The characteristics of the 1,605 participants (46.1% female) who completed the olfactory test (1,283 using 'pen' and 322 using 'paper') are shown in Table 1. On average, 9.1 odours were correctly identified with 'pen', and 9.7 with 'paper'. The distribution of the number of correctly identified odours was significantly shifted to higher numbers when 'paper' was used ( $p < 0.001$ , Figure 1).

Women were significantly more often normosmic than men (57.2% vs. 51.4%,  $p < 0.001$ , Table 2). Both men and women were statistically significantly more often found to be normosmic when comparing 'paper' to 'pen' (62.9% vs. 48.6%,  $p = 0.002$  and 62.9% vs. 48.6%,  $p = 0.002$ , respectively).

No significant differences in the distributions of the four answers between the two methods could be found for seven of the odours (peppermint, banana, liquorice, coffee, clove, rose, and fish) (Table 3). In contrast, identification rates of the 'paper' me-

thod were significantly better when applied to pen 1 (orange), pen 6 (lemon), and pen 10 (pineapple).

A few studies in the past have carried out paper-based odour tests using painted lines on a piece of paper, however the odours were presented exclusively by means of lines drawn on paper<sup>(1,3)</sup>. Their main focus was the test-retest reliability or the determination of normative data and therefore the results within different methods of odour presentation were not compared.

There is only one study, which directly compares pen- and paper-based methods<sup>(2)</sup>. Besser et al.<sup>(2)</sup> used the complete test battery of the 'Sniffin' Sticks 16 test' in 50 volunteers (30 female), where they calculated TDI scores from threshold (T), discrimination (D) and identification (I) testing. In contrast to our results for the identification test, they found no difference between pen-based and paper-based tests for neither the TDI scores nor the identification test. The subjects were asked to

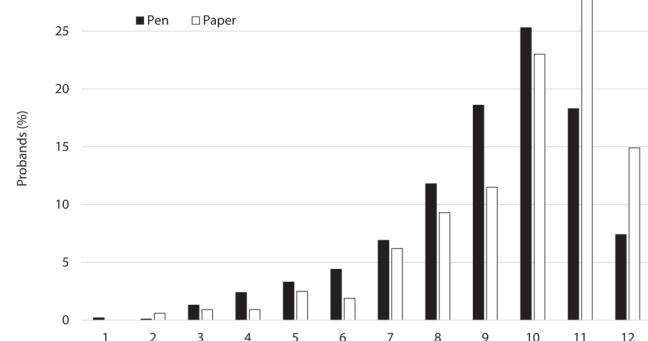


Figure 1. Distribution of the number of correctly identified odours using the 'pen' or 'paper' method. The distribution was significantly shifted to higher numbers when the 'paper' method was used compared with the 'pen' method ( $p < 0.001$ , Mann-Whitney U-test).

Table 1. Participant characteristics.

	Pen n=1283	Paper n=322	Total n=1605	P-value
<b>Sex, no (%)</b>				0.901 <sup>a</sup>
Male	690 (53.8)	175 (54.3)	865 (53.9)	
female	593 (46.2)	147 (45.7)	740 (46.1)	
<b>Age (years), no (%)</b>				0.210 <sup>b</sup>
18-29	11 (0.9)	5 (1.6)	16 (1.0)	
30-39	51 (4.0)	25 (7.8)	76 (4.7)	
40-49	122 (9.5)	19 (5.9)	141 (8.8)	
50-59	197 (15.4)	27 (8.4)	224 (14.0)	
60-69	203 (15.8)	77 (23.9)	280 (17.4)	
70-79	532 (41.5)	140 (43.5)	672 (41.9)	
80-89	167 (13.0)	29 (9.0)	196 (12.2)	
median [IQR]	71.8 [56.6 – 77.3]	70.7 [62.4 – 75.8]	71.6 [57.0 – 77.0]	

<sup>a</sup>  $\chi^2$  test, <sup>b</sup> Mann-Whitney U test

draw curved lines themselves and then to smell the piece of paper, which may have meant that the participant were exposed to the odour for longer than 3-4 seconds, which could influence the results.

In our study, when comparing the recognition rate of odours based on the way they were presented, great differences emerged, where odours on 'paper' identified was 14% higher more normosmics in men and 16% more in women than 'pen'. At this point, we can only speculate why the 'paper' method results in a higher number of normosmics than the usual 'pen'

Table 2. Classification of smell function by sex and method.

	Pen	Paper	Total	P-value (pen vs paper)
<b>Men</b>				0.002 <sup>a</sup>
Anosmia [0-6]	71 (10.3)	9 (5.1)	80 (9.2)	
Hyposmia [7-9]	284 (41.2)	56 (32.0)	340 (39.3)	
Normosmia [10-12]	335 (48.6)	110 (62.9)	445 (51.4)	
<b>Women</b>				0.001 <sup>a</sup>
Anosmia [0-6]	22 (3.7)	7 (4.8)	29 (3.9)	
Hyposmia [7-9]	251 (42.3)	37 (25.2)	288 (38.9)	
Normosmia [10-12]	320 (54.0)	103 (70.1)	423 (57.2)	

<sup>a</sup>  $\chi^2$  test

odorant presentation methods. The effect was highly significant, i.e. the probability of finding this by chance was extremely low.

The better olfactory performance is mainly because the odours orange, lemon and pineapple were better perceived by the participants when presented on 'paper'. These are exclusively fruity smells, which is particularly surprising as the correct identification of the odours lemon and pineapple has in the past caused great difficulties<sup>(4,5)</sup>. Stogbauer et al.<sup>(4)</sup> compared the results of odour testing on 13,825 participants from five major German population studies conducted between 2003 and 2014. In all five studies, the 'Sniffin' Sticks 12 test' was carried out by presenting the pens according to the manufacturer's instructions, and in all five studies, the odours 'lemon' and 'pineapple' were correctly identified far less frequently than all other odours.

Table 3. Percentage of correctly identified odours after using the 'pen' or the 'paper' method.

	Correct Answer (Alternative answers)	Method		P
		Pen (%)	Paper (%)	
Pen 1	Orange (Strawberry, Blackberry, Pineapple)	91.7 (2.4; 3.2; 2.7)	96.0 (0.9; 0.3; 2.8)	0.010
Pen 2	Shoe leather (Smoke, Glue, Grass)	81.4 (3.6; 9.5; 5.5)	77.6 (2.5; 9.9; 10.9)	0.006
Pen 3	Cinnamon (Honey, Chocolate, Vanilla)	52.6 (25.3; 5.4; 16.8)	58.7 (25.2; 2.5; 13.7)	0.050
Pen 4	Peppermint (Chives, Spruce, Onion)	91.4 (0.9; 6.8; 0.9)	92.9 (0.6; 5.0; 1.6)	0.397
Pen 5	Banana (Coconut, Walnut, Cherry)	85.2 (5.1; 4.4; 5.2)	87.9 (3.1; 3.4; 5.6)	0.364
Pen 6	Lemon (Peach, Apple, Grapefruit)	35.2 (10.3; 10.4; 44.2)	57.1 (4.0; 3.7; 35.1)	0.000
Pen 7	Licorice (Gummi bear, Chewing gum, Cookies)	79.1 (8.8; 9.3; 2.8)	80.4 (7.1; 10.2; 2.2)	0.672
Pen 8	Coffee (Cigarette, Wine, Candle Smoke)	88.0 (5.6; 1.8; 4.6)	89.4 (5.3; 1.6; 3.7)	0.892
Pen 9	Clove (Pepper, Cinnamon, Mustard)	85.0 (3.9; 10.0; 1.1)	87.9 (2.5; 9.0; 0.6)	0.482
Pen 10	Pineapple (Pear, Plum, Peach)	54.4 (14.7; 10.5; 20.3)	71.4 (8.7; 5.6; 14.3)	0.000
Pen 11	Rose (Chamomile, Raspberry, Cherry)	87.9 (5.9; 4.4; 1.7)	91.0 (4.0; 4.0; 0.9)	0.385
Pen 12	Fish (Bread, Cheese, Ham)	78.4 (6.5; 10.2; 4.9)	82.0 (3.4; 9.3; 5.3)	0.188

The distinction between the odours 'lemon' and 'grapefruit' seems to pose particular difficulties due to the similarity in chemical composition. In the case of grapefruit the odour originating from natural sources includes limonene, myrcene, linalool and other terpenoid components in addition to the main component nootkatone<sup>(6)</sup>. The olfactory pencils contain high concentrations of a synthetically produced odour, so it is quite possible that the natural odour is slightly distorted and thus not so easily recognised.

Sniffin' Sticks are a widely used, robust, and well-validated psychophysical odour test battery<sup>(7)</sup>. Consequently, changes in the test method that lead to different test results are problematic. On the other hand, the higher number of correctly identified odours with the new test method indicates that there may be a more appropriate way to present suprathreshold odours than using a 'pen'.

A limitation of this study could be that only healthier participants attended the study following the pandemic-related lockdown. However, the Leipzig region was only slightly affected by the first wave of the pandemic and a recent study reported no changes in physical and mental well-being in our study region<sup>(8)</sup>. Summing up, in this study, we show that the presentation of the odours of the "Sniffin' Sticks 12 test" using filter paper leads to a better recognition rate of the odours than using the olfactory pens directly. Should the pen be replaced by the new method in studies or clinical practice, a higher number of participants or patients would likely be classified as normosmic.

### Conflict of interest

There are no competing interests.

### Authorship contribution

KW: Head of LIFE Study Ambulance. Co-PI of the LIFE Adult-Study, Guidance, training and supervision of the ambulance personnel. Responsible for the execution of the examination program, including the sniffing sticks test. Preparation of the manuscript. CE: Co-PI of the LIFE Adult-Study, Preparation of the LIFE Adult-Study protocol and obtaining the ethics vote. Quality assurance and data preparation. Statistical analysis of the research data and participation in the preparation of the manuscript. AH: Member of the LIFE Consortium, Participation in the preparation of the manuscript and discussion of results. ML: Principal investigator of the LIFE Adult-Study. Responsible for the funding. Participation in the scientific discussion of the study results.

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Dr. Kerstin Wirkner, Ph.D.  
Leipzig Research Centre for Civilization Diseases (LIFE)  
University of Leipzig  
Philipp-Rosenthal-Strasse 27  
D-04103 Leipzig  
Germany

Phone: +49 341 9716717

Fax: +49 341 9716719

E-mail:

kerstin.wirkner@life.uni-leipzig.de

This manuscript contains online supplementary material

## SUPPLEMENTARY MATERIAL

### Materials and Methods

#### Study population

This study used data from the first follow-up of the LIFE Adult-Study. The LIFE Adult-Study is a population-based epidemiological study including 10,000 adult citizens aged 18 to 79 years from Leipzig, a city with about 600,000 inhabitants in eastern Germany. The aims of the LIFE Adult-Study are to investigate the prevalence, early onset markers, genetic predispositions, and the role of lifestyle factors of major civilization diseases. The baseline examination, consisting of structured interviews, questionnaires, physical examinations and biosample collection, was carried out between July 2011 and November 2014. Details of the study design have been published elsewhere <sup>(1)</sup>. Since June 2018, a follow-up examination of the study participants in a subset of the baseline population is ongoing. The follow-up examination programme includes olfactory testing using the "Sniffin' Sticks screening 12 test" <sup>(2)</sup>. The study was approved by the ethics committee of the Medical Faculty of the University of Leipzig, and complies with the ethical standards of the Declaration of Helsinki. Written informed consent was obtained from all participants prior to study enrolment.

#### Olfactory measurements

Olfactory function was assessed using the "Sniffin' Sticks screening 12 test" (Burghart, Wedel, Germany). This odour identification test consists of 12 odour-dispensing felt-tip pens with different supra-threshold odours of everyday life (orange, shoe leather, cinnamon, peppermint, banana, lemon, licorice, coffee, clove, pineapple, rose and fish). In this study, odours were presented in two different ways. From June 2018 to March 2020, the test was done according to the manufacturer's instructions. According to these instructions, the participant was first presented with a card with four everyday smells to choose from.

Then, the smelling pen was held for 3-4 seconds in front of both nostrils. This method is hereinafter referred to as 'pen'. After the reopening of the study centre in June 2020 (the study had to be interrupted between March and June 2020 due to the COVID-19 related lockdown in Germany), the test could no longer be performed by holding the pen under the nose due to hygienic reasons. Instead, strips of odourless filter paper of about 8 cm length were used, on one end of which a line of about 2 cm was drawn with the pen. The paper strip was then given to the participant, who smelled it for 3-4 seconds without waving (this method is further termed 'paper'). With both test methods, the participant had to identify the odours by selecting an odour from the four response alternatives presented (forced choice). Depending on the number of correctly identified odours, the participants were classified as "normosmic" (10-12 correct answers), "hyposmic" (7-9 correct answers) and "functionally anosmic" (0-6 correct answers, hereinafter referred to as "anosmic"). This classification is based on the recommendations of the test manufacturer <sup>(2)</sup>. For the present analysis, only participants who have completed the test were included.

#### Statistical analysis

Descriptive statistics were used to characterize the study population. Continuous variables were expressed by means and standard deviations or medians and interquartile ranges (IQR) where appropriate, or by number (proportion) of participants for categorical variables. Between group comparison of continuous variables were done using the Mann-Whitney U-test, categorical variables were compared using the chi-square test or Fisher's exact test, where appropriate. P values <0.05 were considered statistically significant. Statistical analyses were conducted using IBM SPSS Statistics for Windows Version 26 (IBM Corp, Armonk, NY, USA).

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