

## Saccharin Test for the Study of Mucociliary Clearance: Reference Values for a Spanish Population

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**OBJECTIVE:** Mucociliary transport is an important defense mechanism for the airways. The aim of this study was to establish reference values for nasal mucociliary clearance time (MCT) by means of the saccharin test.

**SUBJECTS AND METHODS:** A cross-sectional, descriptive, observational study was performed. The variables studied were nasal MCT, age, and sex. We included 249 healthy nonsmokers (134 male and 115 female subjects) aged over 10 years and stratified the sample by age. To establish reference values, percentiles were calculated and lower and upper limits of normal were established at the 2.5 and 97.5 percentiles, respectively. The association between nasal MCT and the main study variables was then investigated along with the reproducibility of the test.

**RESULTS:** The following values for nasal MCT were obtained for the sample as a whole: mean (SD), 17.17 (8.43) minutes; median (interquartile range), 16 minutes (12-20 minutes), indicating that the central 50% of the sample fell within this 8-minute range; and maximum and minimum values of 4 to 54 minutes (range, 50 minutes). The upper and lower limits of normal were 6 and 36 minutes, respectively. In addition, it is noteworthy that only 6 subjects had a nasal MCT longer than 36 minutes. No significant differences between male and female subjects were found. Nasal MCT showed a positive correlation with age ( $\rho=0.324$ ;  $P=.0001$ ). No significant differences were observed in the reproducibility test ( $P=.208$ ).

**CONCLUSIONS:** In addition to facilitating reliable data on mucociliary function, the saccharin test is easy to do, inexpensive, and reproducible. The data obtained from this study should allow the results of this test to be correctly interpreted for a given subject's age and so facilitate its use in clinical practice.

**Key words:** Saccharin test. Mucociliary clearance. Reference values.

Test de la sacarina en el estudio del aclaramiento mucociliar. Valores de referencia en una población española

**OBJETIVO:** El transporte mucociliar es un importante mecanismo de defensa de las vías respiratorias. El objetivo de este estudio ha sido obtener los valores de referencia del tiempo de transporte mucociliar nasal (TTMCN) mediante el test de la sacarina.

**SUJETOS Y MÉTODOS:** Se ha realizado un estudio observacional, transversal y descriptivo. Las variables estudiadas fueron el TTMCN, la edad y el sexo. Se incluyó a 249 sujetos (134 varones y 115 mujeres) mayores de 10 años, sanos y no fumadores, que se asignaron a grupos según su edad. Para el análisis predictivo de los valores de normalidad se obtuvieron los percentiles, estableciendo los límites de referencia por los percentiles 2,5 y 97,5. Se analizó la asociación entre el TTMCN y las variables principales. Se estudió asimismo la reproducibilidad del test.

**RESULTADOS:** Del análisis global de la muestra destacan los siguientes datos: mediana de 16 min; media  $\pm$  desviación estándar de 17,17  $\pm$  8,43 min; rango de 50 min (4-54); límites de referencia de 6-36 min, y amplitud intercuartílica de 8 min (un 50% central de la muestra entre 12 y 20 min). Es asimismo reseñable que sólo 6 sujetos tuvieron un TTMCN superior a 36 min. No se encontraron diferencias significativas entre varones y mujeres. El TTMCN mostró una correlación positiva con la edad ( $r_s = 0,324$ ;  $p = 0,0001$ ). No se observaron diferencias significativas ( $p = 0,208$ ) en la prueba de reproducibilidad.

**CONCLUSIONES:** El test de la sacarina es fácil de realizar, económico y reproducible, y facilita datos fiables de la función mucociliar. Los datos obtenidos en este trabajo permiten la correcta interpretación de los resultados de esta prueba según la edad del sujeto, lo que facilitará su uso.

**Palabras clave:** Test de la sacarina. Aclaramiento mucociliar. Valores de referencia.

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### Introduction

Mucociliary clearance is a primary defense mechanism of the upper and lower airways and disruption of this process, whether acquired or inherited, predisposes an

individual to chronic nasal, paranasal sinus, and airway infections.<sup>1</sup> While symptoms usually first appear at a young age, they are not particularly specific and diagnosis may be delayed. Further delays may arise because diagnostic techniques for measuring lung mucociliary clearance are complex, expensive, and not widely available. Evidence of a good correlation between tracheobronchial and nasal mucociliary clearance<sup>2,3</sup> allows the use of less complex and invasive techniques, such as the measurement of nasal mucociliary clearance time (MCT) by means of the saccharin test. To use and correctly interpret the results of this technique in everyday clinical practice, normal reference values are needed for the healthy population.

It is important to know whether mucociliary clearance declines with age, as this would theoretically lead to an increased frequency and/or severity of upper and lower respiratory tract infections. Although more infections are indeed observed in older individuals,<sup>4</sup> it is debated whether nasal MCT is related to age; some studies have found an association<sup>5,6</sup> whereas others have not.<sup>7,8</sup> None of these studies has a broad and well stratified sample to allow firm conclusions to be drawn. In addition, most include both healthy individuals and those afflicted by disease of various types, and the number of healthy individuals is small.

Knowledge of the nasal MCT would indicate how values vary according to different factors and conditions of possible clinical relevance: humidity, temperature, exposure to tobacco smoke, environmental pollutants (chronic exposure to polluted air in large cities can disrupt the mucociliary system<sup>9</sup>), oxygen therapy, anesthetics, and respiratory diseases (chronic obstructive pulmonary disease, cystic fibrosis, primary ciliary dyskinesia, bronchiectasis, and asthma). Such knowledge would also help in the assessment of whether therapeutic measures in general are able to have a positive or negative effect on clearance of respiratory secretions.

Several studies have been published on disruption of ciliary function in respiratory diseases, factors that might affect mucociliary clearance, and different methods for its assessment. This contrasts with the lack of studies done in healthy individuals with a large enough sample size to rigorously define the limits of normal of the saccharine test according to age and sex.

The objective of this study was to determine reference values and the upper and lower limits of normal for nasal MCT determined by the saccharine test according to sex and age group in the healthy Spanish population and to assess the reproducibility of the test.

## Participants and Methods

### *Study Design*

This was an observational, cross-sectional, descriptive study. Exclusion criteria were used with a view to limiting the factors that, according to the literature, might influence the variable of interest, that is, nasal MCT. The participants, all of whom were healthy, adequately nourished individuals with no history of substance abuse or bouts of fever, were studied at similar room temperatures and relative humidities and in the same posture. The same investigator undertook all examinations and tried to

minimize possible biological variation by ensuring that relative humidity was between 40% and 70% and that the room temperature was between 18°C and 30°C. The stopwatch was checked from time to time to ensure that it was working properly. The calibration of the spirometer used to assess lung function was also monitored.

The reliability and internal validity was investigated by analyzing the reproducibility of the saccharine test in a second procedure performed in 24 individuals 2 weeks later.

### *Study Sample*

The study sample was drawn from healthy residents of the metropolitan area of Valencia, Spain. These individuals were Spanish nonsmokers aged over 10 years. A nonsmoker was defined as an individual who had never smoked, smoked less than a cigarette a day for less than 6 months, or had not smoked in the 5 years prior to the start of the study. Participants were selected from among hospital workers, school children, patient companions, medical students, members of social and sporting clubs, and health centers for the elderly belonging to the local Social Welfare Department. All individuals participated voluntarily and altruistically in that they did not receive any payment. We excluded individuals with anatomic abnormalities of the upper respiratory tract, history of surgery or nasal trauma, chronic nasal or respiratory disease, acute respiratory tract disease in the 6 weeks prior to the test, altered taste, smokers or those addicted to other drugs, and those receiving pharmacotherapy that might influence mucociliary clearance (antihistaminics, adrenergic agents, anticholinergics, topical decongestants, and mucolytics). Pregnant women were also ineligible for the study. All participants were explained the nature and aim of the test. They signed a written informed consent (or, in the case of participants under 18 years of age, the father, mother, or legal guardian signed the form) and, following the test, they received the results along with an explanation of their clinical significance. During the selection phase, all subjects completed a modified version of the health questionnaire described in the Epidemiology Standardization Project,<sup>10</sup> where the principles for assessing the state of respiratory health were established. For all subjects, a complete medical history was taken and they underwent heart and lung auscultation, anterior rhinoscopy, measurement of exhaled carbon monoxide (nonsmokers were considered as those with levels below 6 ppm), and forced spirometry with a Dataspir 70 511-700-MUI spirometer (Sibel S.A., Barcelona, Spain) according to the method recommended by the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR).<sup>11</sup> Once it had been established that the individual was healthy and did not meet any exclusion criteria, nasal MCT was measured by the saccharine test according to the standard technique described by Andersen et al.<sup>3</sup>

Of the 270 subjects who filled out the respiratory health questionnaire, 251 underwent the test. Nineteen participants met one of the exclusion criteria: 11 had recent nasal or lung disease, 3 were smokers, 3 had deviated nasal septum, and 2 had allergic rhinitis. Two participants decided not to complete the test, and so the final sample comprised 249 individuals.

### *Saccharine Test Method*

A particle of sodium saccharine measuring 1 mm across was placed on the surface of the inferior nasal concha, 1 cm behind its head to avoid the area of squamous epithelium. The participants remained seated with their head tipped slightly forward while breathing normally (not forced), without sneezing or blowing their nose, and without taking any substances that might interfere with the test. They were told to indicate when they noted any

particular taste. The actual taste they were to expect was not specified in order to avoid false positives. The saccharine particle was carried by means of ciliary transport along the entire nostril until it reached the oropharynx, whereupon a characteristic sweet taste could be perceived. The time elapsed was recorded to the nearest minute and the test was considered complete. If the participant did not detect any taste after 60 minutes, a saccharine particle was placed on the tongue to ensure that he or she did not suffer any taste abnormalities. The most patent nostril with least resistance to physiological airflow was chosen.

The Department of Pharmacy and Pharmaceutical Technology of the Faculty of Pharmacy of the University of Valencia supplied the saccharine particles. Uniformity of particle size (1 mm) was achieved by electronic screening. The apparatus needed to perform the test is very rudimentary and consists of a head mirror with light source, a 2-blade speculum to open the entrance to the nose, some curved tweezers to place the particle in the nostril, and a stopwatch.

#### Assignment to Groups

Three age groups were established. These were balanced in terms of the number of subjects and age ranges: group 1, aged 10 to 29 years (84 participants, 44 male participants and 40 female participants); group 2, aged 30 to 49 years (85 participants, 50 men and 35 women); and group 3, aged over 50 years (80 participants, 40 men and 40 women).

#### Data Management and Statistical Analysis

The database of the SPSS program (SPSS Inc, Chicago, Illinois, USA) was used. Once data collection was complete, the Kolmogorov-Smirnov test was used to determine how closely the data followed a normal distribution. The degree of deviation from normal distribution in our study ( $P=.009$ ), even after logarithmic transformation, indicated that nonparametric statistical tests were required. Thus, the Mann-Whitney U test, the Wilcoxon T test, and the Kruskal-Wallis test were used for comparisons and Spearman correlation coefficients ( $\rho$ ) were calculated. Initially, the data were analyzed for male and female participants separately, with age and sex as independent or predictive variables and nasal MCT as the dependent variable. On finding that there was no correlation between sex and nasal MCT, the different groups were studied solely according to age. Given that linear regression analysis to produce reference values was not possible because the distribution was not normal, the 2.5th, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97.5th percentiles were calculated. There is consensus that the reference range should contain the central 95% of the distribution of the reference population.<sup>12</sup> Thus, the reference limits were estimated as the 2.5th and 97.5th percentiles, discarding the upper and lower 2.5%.<sup>13</sup> Quantitative variables were also described by medians and percentiles, as these statistics would not be affected by outliers. The dispersion was characterized by maximum and minimum values, range, and interquartile range (which contains the central 50% of the individuals of the distribution). The analysis was completed with descriptive statistics (mean, median, maximum and minimum values) and percentile distributions for each age and sex group. Possible associations between the result of the nasal MCT test and these 2 variables were investigated.

The reproducibility of the saccharine test was investigated by conducting a second procedure in 24 individuals 2 weeks later. The differences between the values obtained in the initial test and the second test were measured and compared using the Wilcoxon T test. Statistical significance was set at  $P<.05$ .

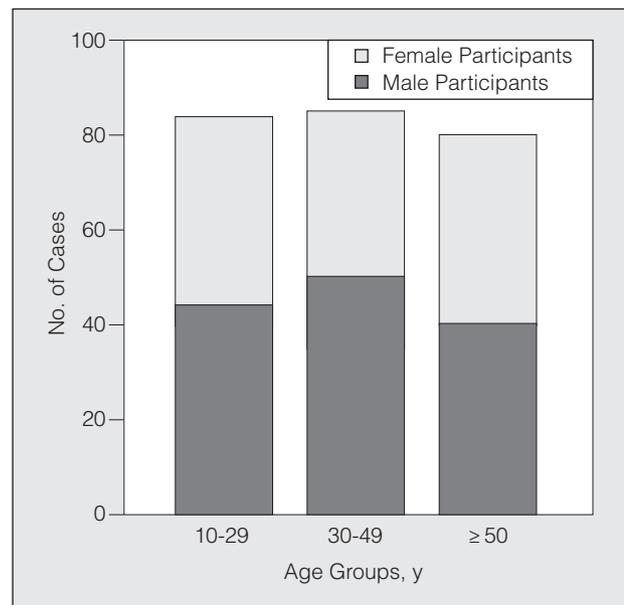


Figure 1. Distribution of the sample by age and sex groups.

## Results

The study included 249 subjects—134 men (53.8%) and 115 women (46.2%)—aged between 10 and 83 years (range, 73 years). Figure 1 shows the age distribution of the sample by age and sex.

#### Descriptive Statistics for Nasal MCT

In the overall sample the median nasal MCT was 16 minutes and measures were widely dispersed, with a range of 50 minutes. A nasal MCT greater than 36 minutes was reported for 6 participants, that is, 2.4% of all subjects studied (97.5th percentile). Individuals in the central 50% of the distribution had nasal MCTs of between 12 and 20 minutes (that is, the interquartile range was 8 minutes). No significant differences in nasal MCT were found between male and female participants (Mann-Whitney U test,  $P=.786$ ). Both groups had the same median time (16 minutes) and interquartile range (8 minutes), although the actual range was greater in male than in female participants (50 minutes vs 31 minutes, respectively).

#### Nasal MCT and Age

In the overall sample, nasal MCT and age were positively correlated ( $\rho=0.324$ ,  $P<.001$ ). The scatter plot (Figure 2) revealed a broad dispersion, but with a trend towards higher values with increasing age.

Tables 1 and 2 show the data for nasal MCT for the 3 age groups. Of note is that the median value of 15 minutes is the same for the first 2 groups (10-49 years), and significantly different for the third group (median, 18 minutes). A breakdown of the data by percentiles showed that 95% of the individuals studied had times between 4.1 and 29.6 minutes for the group aged 10 to 29 years;

TABLE 1  
Descriptive Statistics of Nasal Mucociliary Transport Time (in Minutes) by Age Group

Age Range, y	No.	Mean (SD)	Median	Minimum	Maximum	Max-Min	p75-p25
10-29	84	15.01 (6.21)	15	4	31	27	7
0-49	85	15.67 (7.47)	15	4	46	42	9
≥50	80	19.75 (7.58)	18	9	54	45	7.75

Abbreviations: p25, 25th percentile; p75, 75th percentile.

TABLE 2  
Nasal Mucociliary Transport Time Percentiles by Age Group

Age Range, y	Percentiles								
	2.5 th	5 th	10 th	25 th	50 th	75 th	90 th	95 th	97.5 th
10-29	4.13	5.25	7	11	15	18	23.50	26	29.63
30-49	6	7	8	10	15	19	23	30.80	42.95
≥50	10.03	11	12	15	18	22.75	27.80	35.95	45.75

between 6 and 42.95 minutes for the grouped aged 30 to 49 years; and between 10.03 and 45.7 minutes for the group aged 50 years or more.

#### Reproducibility of the Saccharin Test

The analysis of the results obtained on repeating the test in 24 subjects showed a nonsignificant 1-minute difference in the medians ( $P=.208$ , Wilcoxon T test).

#### Discussion

Analysis of mucociliary clearance can provide grounds for suspected diagnosis of certain diseases and help assess the consequences of certain conditions and effects of certain treatments. In primary ciliary dyskinesia, early diagnosis can ensure that appropriate treatment is introduced as soon as possible, thereby slowing the progressive decline in pulmonary function.<sup>14</sup> It has been found that tests that employ saccharin or similar substances (aspartame) are useful for scientific investigation, easy to perform, without the need for sophisticated equipment, and do not cause the subject discomfort. Although such tests depend on a subjective factor, they do give a well-defined measure of nasal MCT, as individuals can readily perceive the sweet taste. The published results for mucociliary clearance, determined by measuring transport time of different substances, rate of transport, and ciliary beat frequency, have varied widely. The discrepancies may be explained by the range of methods used to study this process. The most sensitive and specific may be the most complex and least widely available, whereas the methods that are easiest to perform might not be sufficiently sensitive or specific. The method used in this study, the saccharine test, was introduced by Andersen et al<sup>3</sup> in 1974 and since then has been employed in many studies as a method for assessing

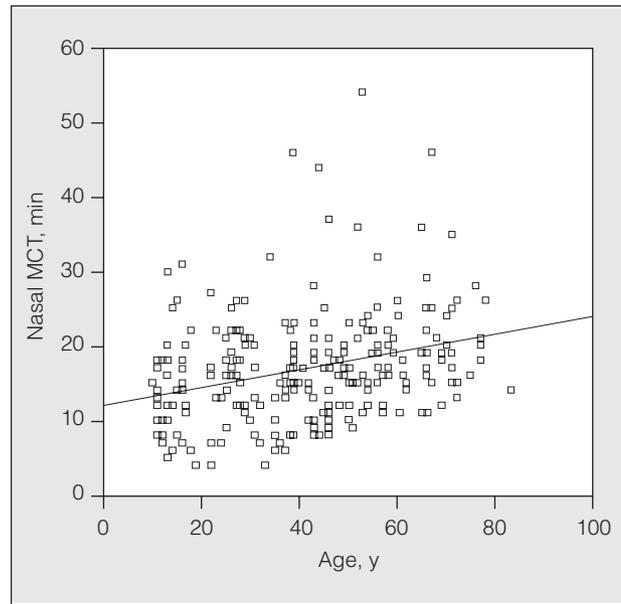


Figure 2. Scatter plot of nasal mucociliary transport time (MCT) against age.

mucociliary clearance. The correlation between nasal and tracheobronchial mucociliary clearance rates has been well established,<sup>2,3</sup> underlining the utility of studying nasal mucociliary transport for investigating overall mucociliary function without resorting to invasive methods. Some investigators criticize the use of saccharine particles for determining mucosal transport,<sup>15,16</sup> arguing that the solubility of the particle in mucosal secretions means that the test does not truly reflect the transport of inert particles deposited in the respiratory tract. These authors propose using charcoal powder. Nevertheless, there are studies that show a good correlation between nasal MCT measured with the saccharine test and ciliary beat frequency measured by photometry,<sup>17,18</sup> and a significant negative correlation with the transport rate measured with technetium-99m-tagged resin particles.<sup>19</sup> Transport times of charcoal powder and saccharine are also correlated, although when saccharine is used, the times observed are longer.<sup>15</sup> Moreover, the use of charcoal powder requires regular examination of the nasopharynx to detect the arrival of particles, whereas when saccharine is used, the patient indicates when he or she notes a sweet taste.

In his doctoral thesis, Coromina Isern<sup>20</sup> compared 3 methods for measuring nasal mucociliary clearance (saccharine test, methylene blue test, and transport rate measured with radioactive isotopes) in patients with allergic rhinitis and nasal pollinosis, concluding that the saccharine test is the simplest, most reliable, fastest, and least expensive.

Given the nonnormal distribution of measurements of the dependent variable in our study, we used medians, ranges, and percentiles as the most appropriate means of defining the central tendency and dispersion. The median better represents the central tendency than the mean, and the first and third quartiles (25th and 75th percentiles, respectively), provide a natural indication of the variability

of nasal MCT for individuals with values in the central 50% of the distribution. Percentiles can be used to establish the reference limits.

This study may be subject to a selection bias in that the sample comprises volunteers who are not randomly selected. Nevertheless, given that participants were healthy, as determined prior to enrollment in the study and subsequent filtering for exclusion criteria, it is possible to argue that the sample studied represents the target reference population without affecting external validity. Analyzing reproducibility by retesting the same persons, and using a single tester to conduct and interpret the test contributed to the internal validity of the study. In addition, electronic screening of the size of the saccharine particles guaranteed their homogeneity.

The descriptive statistics (medians, ranges, and percentiles) used to generate the reference values are easy to interpret in clinical practice, facilitating the rigorous establishment of normal reference values. The limits of normal correspond to the values at the 2.5th and 97.5th percentiles, with the values lying within those limits comprising the range of reference.

The sex of the individual is an important predictor in various laboratory tests, and many investigators have studied the influence of sex on mucociliary clearance. Most agree that there are no significant differences between men and women.<sup>6,21-23</sup> In this study, the indicators of the central tendency of distributions were very similar for both sexes, with no significant differences. The only difference of note was the larger range of values in male participants.

In contrast, the results published to date do not agree on the influence of age on the mucociliary transport system. We should note that published studies have not been based on large samples of healthy individuals. In Spain, the largest study of the saccharine test published to date is that of Jolis et al,<sup>5</sup> who included 20 healthy individuals in each of 3 age groups (20-35, 35-45, and 55-65 years). According to the findings of that study, nasal MCT increased with age, with significant differences between age groups. The study with the largest sample size, but restricted to children, was that of Sánchez,<sup>24</sup> who assessed mucociliary transport in 150 healthy children using charcoal powder. Given that only children were included in that study, it is impossible to draw any conclusions on the influence of age on mucociliary clearance. Hellín Meseguer and Merino Gálvez,<sup>22</sup> for their part, used charcoal powder to study nasal MCT in 132 healthy individuals in 4 age groups. However, only 28 subjects were aged over 40 years. They reported that mean time was greater in older age groups, although the differences were not significant. Sakakura et al<sup>23</sup> did not observe any differences in individuals aged under 60 years, in a study of nasal MCT using saccharine grains in 127 volunteers without nasal or sinus disease. They did not indicate which criteria were used to ensure that the individuals included were healthy, only mentioning that they did not suffer any local disease. The saccharine grains that they used were not of a homogeneous size and were larger than those usually employed. In view of these methodological shortcomings, it is impossible to draw valid conclusions from that study.

Other authors<sup>7,8,21</sup> who have studied mucociliary function in healthy individuals by analyzing ciliary beat frequency have also failed to find any correlation with age. Aguis et al<sup>7</sup> and Kao et al<sup>21</sup> included fewer than 60 subjects in their studies, and Jorissen et al<sup>8</sup> had a sample size of 113 but only 16 were aged over 30 years. The most recent study to analyze the effect of age on nasal mucociliary clearance examined nasal MCT using the saccharine test, ciliary beat frequency using photometry, and ciliary ultrastructure using electron microscopy.<sup>6</sup> The investigators performed the saccharine test on 43 of the total sample of 90 individuals, observing that age was associated with decreased ciliary beat frequency, increased nasal MCT, and greater prevalence of ultrastructural ciliary abnormalities. Our study, with 249 healthy individuals evenly distributed according to age, showed a positive correlation ( $\rho=0.324$ ;  $P<.001$ ) between age and nasal MCT measured with the saccharine test. Several authors include the term "slow clearers" in the results of their studies of mucociliary transport,<sup>22,25,26</sup> a term which is applied to those whose clearance time differs from average without any apparent disease that might justify slow clearance times. The percentage of such individuals ranges from 20% to 1.33%.<sup>27</sup> In our study, only 6 of 249 subjects had a nasal MCT greater than 40 minutes, that is, 2.4% of all individuals. There seems to be agreement in that individuals with a nasal MCT greater than 60 minutes should be studied to rule out any disease of the mucociliary system.<sup>21</sup> In our opinion, mucociliary dysfunction should be ruled out in those individuals with nasal MCT above the 97.5th for their age group.

Assessment of ciliary function can be carried out with simple and inexpensive equipment, and the skills needed for the test can be quickly acquired. The study of mucociliary transport should be an essential part of the workup of patients with bronchiectasis or recurrent respiratory infections, particularly if primary ciliary dyskinesia is suspected. The saccharine test could be useful as a screening method for detecting diseases with abnormal mucociliary clearance and for assessing the repercussion of certain treatments on mucosal transport. With the findings of this study, it is reasonable to recommend the use of the saccharine test in the early stages of the study of mucociliary function, before resorting to more sophisticated techniques that are less widely available and more expensive, such as ciliary beat frequency and isotope-based methods. In addition to providing reliable information on mucociliary function, the saccharin test is easy to do, inexpensive, and reproducible. The data obtained in this study will assist in the appropriate interpretation of the results of this test, according to age of the individual, thus facilitating its systematic use.

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