

Exhaled Breath Condensate: Standardized Collection of Samples From Healthy Volunteers

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Expired breath condensate collection is a noninvasive technique for obtaining a sample in which to analyze substances that reflect the functional status of the lung and other tissues. Twenty healthy volunteers provided 3 expired breath samples: the second was collected 20 minutes after the first and the third 48 hours after the first. The air and condensate volumes were assessed. The mean (SD) volume of condensate in exhaled air over a period of 15 minutes was 1.8 (0.5) mL (95% confidence interval [CI], 1.5-2 mL) and the coefficient of variation was 29%. Analysis of variance in the 3 samples demonstrated no significant differences. The mean volume of air inhaled over 15 minutes was 119 (25) L (95% CI, 112-125 L). These results indicate that it takes at least 15 minutes and the inhalation of some 120 L of air to collect a condensate volume that exceeds 1.5 mL, sufficient to allow distribution in aliquots to analyze fundamental physical and chemical properties (conductivity, pH) and certain relevant biomarkers.

Key words: *Exhaled breath condensate. Inflammation. Noninvasive technique.*

Condensado de aire espirado: estandarización de la recogida de muestras en voluntarios sanos

El condensado de aire espirado (CAE) es una técnica no invasiva que permite la obtención de un conjunto de sustancias que expresan el estado funcional del pulmón y otros tejidos. A 20 voluntarios sanos se les realizó un total de 3 pruebas a cada uno: una primera recogida, seguida de otra a los 20 min y la última a las 48 h de la inicial. Al final de la prueba se estimaron el volumen de muestra de condensado obtenida y el volumen de aire movilizado. La media (\pm desviación estándar) del volumen de muestra de CAE recolectado en 15 min fue de $1,8 \pm 0,5$ ml, con un intervalo de confianza (IC) del 95% de 1,5 a 2 ml y un coeficiente de variación del 29%. El análisis de la variancia entre las 3 muestras analizadas no mostró diferencias estadísticamente significativas. La media del volumen de aire inspirado en los 15 min fue de 119 ± 25 l, con un IC del 95% de 112 a 125 l. Nuestros resultados indican que para obtener un volumen de muestra superior a 1,5 ml son necesarios al menos 15 min de recogida y haber movilizado unos 120 l de aire, lo que permite la distribución en alícuotas para analizar los componentes físicos y químicos básicos (conductividad, pH) y ciertos biomarcadores de interés.

Palabras clave: *Condensado de aire espirado. Inflamación. Técnica no invasiva.*

Introduction

Exhaled air consists basically of water vapor and substances that reflect the functional status of the lung and other tissues. These substances (both volatile and nonvolatile) can be analyzed by condensing exhaled air by passing it through a cooling unit. The collection of exhaled breath condensate (EBC) is a simple noninvasive technique that requires minimal cooperation

on the part of the patient (of particular interest for use with pediatric and geriatric patients), is reproducible over time, has short collection periods, does not require special facilities or specially trained staff, and can be performed with portable equipment and at a low cost.¹ The procedure was initially developed in the 1980s in the former Soviet Union^{2,3} but became widely known through the work of the team of Kharitonov and Barnes⁴ in the late 1990s. The procedure was introduced in Spain a short time ago and there has been particular interest in standardizing sample collection.⁵

EBC has raised expectations in the study of inflammatory lung disease despite a lack of sufficient evidence concerning the standardization of sample collection and the validation of the analysis of biomarkers in the samples of either healthy individuals or patients with pulmonary or systemic diseases. The search

This study was financed by Red Respira-ISCIH-SEPAR.

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Manuscript received September 4, 2004. Accepted for publication January 12, 2005.

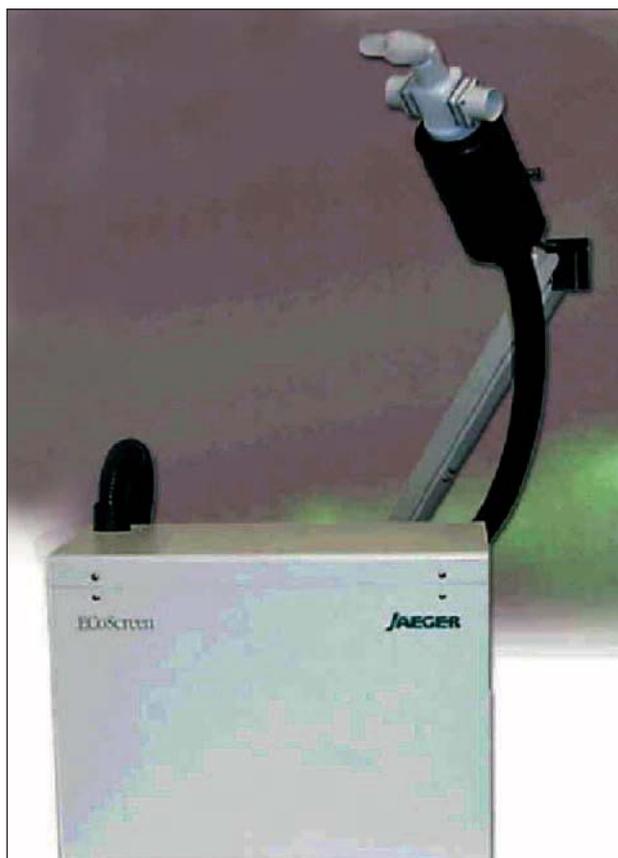


Figure 1. EcoScreen system (mouthpiece, connector, and cooling unit in the upper part of the figure) (Jaeger, Hoechberg, Germany).

for an immediate clinical application for the procedure has led to a degree of confusion with respect to methods of collecting, storing, and processing EBC samples.

The objective of the present study was to validate the procedure for collecting EBC samples in healthy individuals, by comparing intraindividual differences in samples taken from each subject upon collection and after 48 hours, and to establish the bases for a sample collection procedure for use in our pneumology department.

Material and Methods

We studied 20 healthy volunteers who freely accepted to participate in the study. Inclusion criteria were age over 18 years with no previous history of lung disease and normal physical examination. Exclusion criteria were active smoker or known chronic disease. The protocol was approved by the hospital ethics committee.

Protocol for Collecting EBC in Healthy Volunteers

Sample collection was carried out in a single laboratory with a temperature of 20°C to 25°C and relative humidity of 50% to 60%. The volunteers had taken no food in the 2 hours preceding collection and had received no medication in the preceding 24 hours. Samples were collected with the EcoScreen (Jaeger, Hoechberg, Germany) (Figure 1). The Wright Spirometer Mark 8 (Ferraris, Enfield, UK) was used

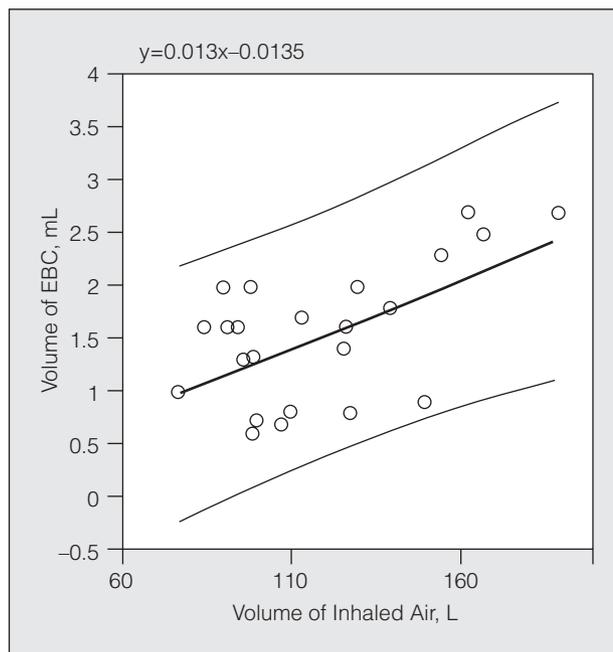


Figure 2. Linear regression of the relation of exhaled breath condensate (EBC) and volume of inhaled air (L).

to measure volume of inhaled air. Each subject provided 3 expired breath samples: the second was collected 20 minutes after the first, and the third 48 hours after the first.

The protocol was as follows:

- Start up the system.
- Connect the mouthpiece to the cooling unit.
- Wait 20 minutes for the temperature of the cooling unit to go down.
- Instruct the subject to clean oral cavity with distilled water, rinsing and spitting out repeatedly.
- Seat the subject comfortably.
- Apply mouthpiece and nose clips.
- Connect volume-measuring device to the inhalation valve.
- Instruct subject to breathe slowly.
- Begin the 15-minute count.
- Measure volume of inhaled air every 3 minutes.
- At minute 6, remove the nose clips, have the subject rest and spit out accumulated saliva.
- Begin measuring again until minute 15.
- Measure volume of resulting sample.
- Proceed to separate the sample into aliquots.
- Freeze the sample at -80°C.

Results

The mean (SD) age of the 20 subjects (15 men and 5 women) who participated in the study was 39 (9) years; mean height, 167 (8) cm; and mean weight, 71 (11) kg.

The mean volume of condensate in exhaled air over a period of 15 minutes was 1.8 (0.5) mL (95% confidence interval [CI], 1.5-2 mL) and the coefficient of variation was 29%. Analysis of variance in the 3 samples demonstrated no significant differences. The mean values for each collection period are shown in the Table.

TABLE
Volume of Condensate in Exhaled Air Obtained
in the 3 Samples

	First	Second	Third
Mean (SD), mL	1.8 (0.5)	1.8 (0.5)	1.7 (0.5)
95% confidence interval	1.5-1.9	1.5-2	1.5-2
Coefficient de variation, %	29	30	30

The mean volume of air inhaled over 15 minutes was 119 (25) L, (95% CI, 112-125 L). The direct relation between volume of collected EBC and volume of air inhaled for each individual (Figure 2) is expressed by the following equation:

$$\text{Volume of EBC (mL)} = 0.013 \text{ volume of air (L)} + 0.255. \\ (\text{R}=0.6; P<.0001.)$$

Discussion

Our results indicate that it takes at least 15 minutes and the inhalation of some 120 L of air to collect a condensate volume that exceeds 1.5 mL, sufficient to allow distribution in aliquots to analyze fundamental physical and chemical properties (conductivity, pH) and certain relevant biomarkers.

No significant differences were observed between the 3 samples and the volume of condensate obtained was directly related to volume of air inhaled.

In recent years, several published studies have dealt with the clinical application of EBC, chiefly in the area of pulmonary inflammation.¹ Attention has been called repeatedly to the need to standardize the procedure, and to date, it remains insufficiently described and validated reference values remain unavailable.⁶

Another issue to bear in mind is that of obtaining a sample that contains a rich concentration of biomarkers. This, considered in the context of sample dilution, might lead to future modifications in the procedure, such as enriching the sample through the inhalation of humidified air or concentrating the sample by means of lyophilization.^{7,8}

There are several mechanisms by which volatile and nonvolatile substances pass from the lungs to EBC. In the first place, the turbulence that develops as air passes generates sufficient energy for molecules to break away from airway walls. Then, the concentration of some of the most frequently analyzed substances in EBC (like hydrogen peroxide) depends on the airflow generated during the procedure, while that of other components (8-isoprostane and ethanol) is independent of airflow.¹ Another mechanism affecting the collection of EBC is the Venturi effect produced with the opening and closing of the bronchi and alveoli, although little is known of its implications.¹

Another variable to be taken into consideration in the standardization of the procedure is the diversity of devices available. The 2 devices available in Spain are EcoScreen, the system used in the majority of international studies and in ours, and the Anacon prototype (Biostec, Valencia, Spain), used by several groups of researchers. Any such system must be able to generate a sufficient sample of condensate for analysis and have external controls for temperature and humidity. It would also be desirable for such systems to allow the measurement of ventilatory parameters, as these can affect final sample volume. Airflow can also modify the type of biomarkers contained in the EBC. These options are not included in any of the systems currently on the market, and separate modules must be acquired to measure ventilatory parameters. In the absence of such measuring devices, determining collection time is a valid strategy to ensure an acceptable EBC volume. It is also important that the material used be inert, convenient, and easy to handle and clean.

The effect of nasal and saliva contamination on the final sample and the need to use nose clips during collection have not been studied sufficiently. Studies on the variability and reproducibility of the analysis of most substances are also lacking and the clinical interpretation of many of the findings has not been sufficiently verified.¹

The method for obtaining EBC samples requires at least 15 minutes or the inhalation of enough air to generate a sufficient volume of condensate. The reproducibility of the procedure to generate such a volume is satisfactory. Further studies considering different variables are needed to complete the standardization of the procedure.

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