

Short communication

Comparison of two methods for exhaled breath condensate collection

Background: Exhaled breath condensate (EBC) is a noninvasive method to obtain samples from fluids lining the respiratory surfaces. Even though various methods and devices are now available, the relative efficiency of these methods for recovering airway mediators and characterizing EBC has not been established.

Aim: To compare the volume, pH, lipid mediator, and protein concentrations in EBCs collected by two commonly used commercially available devices, RTube™ and ECoScreen.

Methods: Exhaled breath condensates were obtained consecutively using the RTube™ and ECoScreen methods from 30 healthy, nonallergic adults. Samples were immediately placed on dry ice after collection. pH was measured after argon deaeration. Cysteinyl leukotrienes (cys-LTs) were measured as a representative lipid mediator and eotaxin as the protein mediator by using enzyme-linked immunosorbent assay.

Results: The mean volume of samples obtained with ECoScreen ($1880 \pm 116 \mu\text{l}$) was significantly higher than that obtained with RTube™ ($1405 \pm 82 \mu\text{l}$) ($P < 0.001$). Concentrations of both cys-LTs [205.4 pg/ml ($65.5\text{--}472.3$) with ECoScreen vs 21.6 ($11.87\text{--}152.2$) with RTube™, $P < 0.001$] and eotaxin [17.0 pg/ml ($11.4\text{--}22.4$) with ECoScreen vs 11.7 ($10.5\text{--}13.5$) with RTube™, $P = 0.01$] were significantly higher in samples collected with ECoScreen than with RTube™. There was no significant difference between the pH measurements.

Conclusion: Compared with RTube™, collection of exhaled breath by ECoScreen allows larger volumes to be collected and detects protein and lipid mediators with greater sensitivity. These differences in mediator recovery may be due to the differences in the collection temperature.

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There has been an increasing interest in the use of exhaled breath condensate (EBC) as a noninvasive method to investigate lung diseases (1, 2). However, there is no standard method for EBC collection and the use of various devices renders the comparison of different studies difficult. The importance of studies using custom-designed devices for EBC collection (2, 3) has led to the production of commercially available devices of more standard design. However, relative efficiencies of these devices regarding recovery of EBC or airway mediators are unknown (2).

Two commonly used commercial systems for EBC collection are RTube™ (Respiratory Research Inc., VA) and ECoScreen (Jaeger, Wurzburg, Germany) (3). As understanding the efficiency of these devices is fundamental to defining their sensitivity and for comparison of results across studies, we aimed to compare the efficiency of these two devices for collecting EBC and preserving mediators. For this purpose, we measured the pH, the volume, a representative class of lipid mediators [cysteinyl leukotrienes (cys-LTs)] and a protein mediator (eotaxin)

in contemporaneous samples collected with ECoScreen and RTube™ from the same individuals.

Methods

Thirty healthy, nonsmoking adults served as the subjects of this study. The subjects had never had a physician's diagnosis of asthma, never smoked and denied respiratory symptoms on a standardized questionnaire. All individuals had normal lung function ($\text{FEV}_1 > 80\%$ predicted) and not more than 5% increase after inhalation of $200 \mu\text{g}$ of salbutamol. Epidermal skin tests with common aeroallergens were negative. None of the subjects had an episode of upper or lower airway infection in the 4 weeks prior to the study or received any topical or systemic medication during this period. The Ethics Committee of Hacettepe University approved all study procedures and each subject provided written informed consent.

Study design

Exhaled breath condensate collection was performed by both the ECoScreen and RTube™ methods in random sequence but consecutively and within 1 h. All studies were performed in the morning

by individuals who refrained from consumption of food and beverages for 2 h before the measurements. The subjects rinsed their mouth with water and breathed at normal frequency and tidal volume through the mouthpiece for exactly 10 min. After collection, EBC samples were immediately aliquoted into separate tubes on dry ice, and stored at -80°C until analysis. All samples were collected within 15 days of one another and assayed together after thawing.

EBC collection

ECoScreen In this collection system, exhaled air enters and leaves the chamber through a two-way, nonbreathing valve in which inspiratory and expiratory gas admixture is prevented and saliva filtered. Expiratory air flows through a lamellar condenser that is surrounded by a cooling cuff. The interior temperature is -15°C or cooler.

RTubeTM In this system, EBC is collected using a disposable collection kit. RTubeTM has a mouthpiece connected through a unique one-way valve into a polypropylene collection tube surrounded by an aluminum sleeve that is cooled to -20°C before sample collection. The circuit contains a saliva trap. The temperature profile inside the cooling sleeve was measured with a calibrated thermometer (BioTemp, Allafiance, Chemillé, France) as a function of time after removal from the -20°C freezer. Five observations were made over the 10-min interval that sample collection is recommended.

EBC analysis

Volume Exhaled breath condensate was immediately separated into 130 μl aliquots with calibrated pipettes (Pipetman 200 and 10 μl ; Gilson International, Roissy, France).

pH The pH was measured using a pH meter (Beckman Φ 34; Fullerton, CA, USA) after argon gas was passed over the sample at 2 l/min for 10 min.

Measurement of mediators Cysteinyl leukotriene concentration was measured by using an enzyme-linked immunosorbent assay (ELISA; Cayman Chemicals, Ann Arbor, MI, USA). This kit measures all cys-LTs (LTC_4 , LTD_4 , and LTE_4) in one assay. The intra-assay and inter-assay coefficient of variability were within 10% and detection limit of this assay was 14 pg/ml. Eotaxin was measured with a commercially available ELISA (Biosource International, Camarillo, CA, USA). The inter-assay coefficient of variation was less than 10% and the detection limit of the assay was 8 pg/ml.

Statistical analyses

Statistical analysis was performed with SPSS 11.5 for Windows (SPSS Inc., Chicago, IL, USA). Data are presented as mean $\pm$ standard error of the mean or as median and interquartile ranges as dictated by the normalcy of the data distribution. Volumes of EBC were compared with paired-samples *t*-test and the pH and the mediator levels by Wilcoxon signed rank test. $P < 0.05$ was considered significant.

Results

Exhaled breath condensate was successfully collected in all subjects with both RTubeTM and ECoScreen. The temperature inside RTubeTM increased dramatically

2 min after transfer from -20°C (a freezer) into room air and continued to warm during the collection process (Fig. 1). Mean volume of EBC samples obtained with ECoScreen ($1880 \pm 116 \mu\text{l}$) was significantly higher than that collected with RTubeTM ($1405 \pm 82 \mu\text{l}$) ($P < 0.001$) (Fig. 2A). Concentrations of both cys-LTs [205.4 pg/ml ($65.5\text{--}472.3$) vs 21.6 ($11.9\text{--}152.2$); $P < 0.001$] and eotaxin [17.0 pg/ml ($11.4\text{--}22.4$) vs 11.7 ($10.5\text{--}13.5$); $P = 0.01$] were significantly higher in samples collected with ECoScreen than those collected with RTubeTM. No significant difference was shown between median pH values measured with the two collection methods [7.55 ($6.88\text{--}7.90$) with ECoScreen vs 7.54 ($7.09\text{--}7.93$) with RTubeTM, $P = 0.419$] (Fig. 2D).

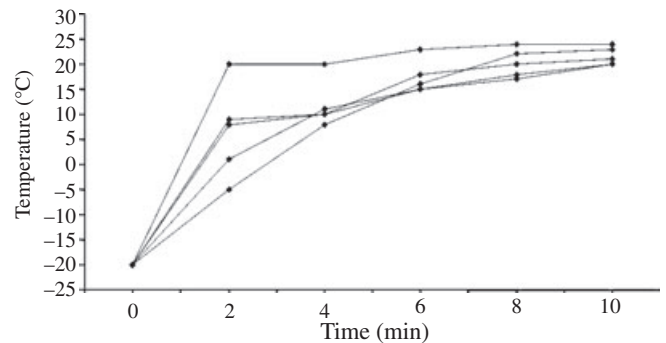


Figure 1. Temperatures recorded within RTubeTM. Time zero refers to the time point immediately before the commencement of exhaled breath condensate collection when RTube is taken out of a -20°C freezer.

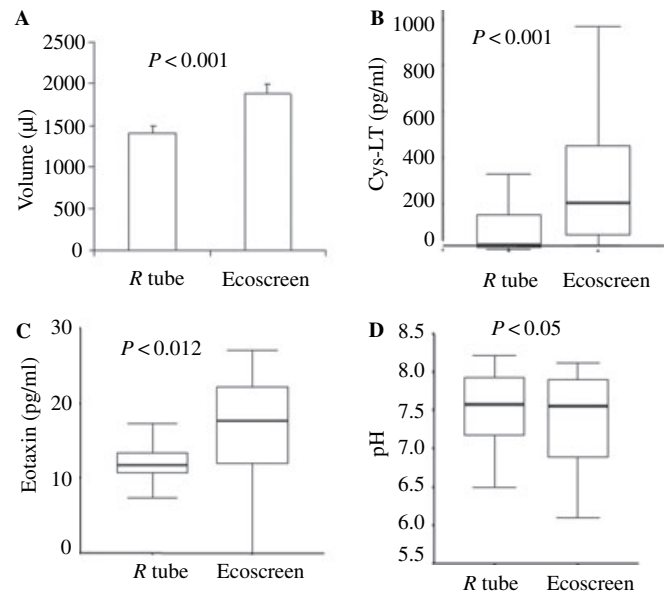


Figure 2. Comparison of (A) volumes, (B) cysteinyl leukotriene levels, (C) eotaxin levels, and (D) pH of exhaled breath condensate collected with RTubeTM and ECoScreen. The volume is presented as mean $\pm$ SEM and the remaining measurements as median and interquartile ranges.

Discussion

The ECoScreen method recovered significantly higher volumes and more efficiently detected lipid (cys-LT) and protein (eotaxin) mediators. pH measurements were similar with both methods (4).

To reduce confounding by differences in the conditions under which samples were collected, we minimized the effect of diurnal variation, ambient temperature, and atmospheric pressure by sampling each subject by both methods within one morning hour. We minimized mediator degradation by immediately aliquoting the samples in tubes stored on dry ice and measuring the levels immediately after the first thaw of the sample. The only variable that differed between the two techniques was the type of collection device.

The ECoScreen method uses an electrical refrigeration device that conducts exhaled air through a lamellar condenser with a sample collection vial inserted into a cooling cuff. The RTubeTM is a portable device consisting of a re-usable aluminum sleeve placed over a disposable vertical condensation tube and brought into thermal equilibrium with a -20°C freezer. After removal from the freezer the apparatus is connected to two uni-directional valves and a mouthpiece. One significant difference between these devices is the temperature inside the cooling sleeve and cooling cuff to which the condensate is exposed. The temperature in the ECoScreen cooling cuff is maintained at $\leq -15^{\circ}\text{C}$ resulting in a temperature of -2 to 0°C within the sample collection chamber. In contrast, we found that the temperature within the RTubeTM cooling increases to ambient temperatures over the recommended time course of a condensate collection maneuver. Moreover, condensate collection occurs on a duckbill valve fixed at the nearest point to the mouth and thus has the highest temperature in the circuit. These higher temperatures have the capacity to lead to degradation and more rapid loss of heat labile substances in the condensate. Consistent with this difference, we found that the concentrations of both cys-LT and eotaxin were lower when the RTubeTM method

was used. This observation is compatible with the known short half-life of cys-LTs stored at physiological temperatures (5) and the importance of low temperatures for their preservation in biological samples (6). The difference in the recovery of eotaxin between the methods was less dramatic, consistent with its greater thermal stability.

The collection chambers of both systems are made of polypropylene, but there are differences in the materials that could affect sample recovery. The main difference between the two systems is that the duckbill valve of RTubeTM is made of silicone rubber whereas ECoScreen's lamellar condenser is made of aluminum coated with teflon. It is possible that some mediators may be recovered more efficiently from Teflon surfaces including the leukotrienes measured in this study.

The concentration of cys-LT reported by Bucchioni et al. using ECoScreen was 27.2 pg/ml for healthy adult subjects (7) and is much lower than the levels that we report. These differences do not appear to be related to the type of immunoassay used. Consistent with the thermal sensitivity of cys-LT, freezing our samples on dry ice immediately after collection and avoidance of repeated freeze-thaw cycles may have caused this difference. Our study is compatible with a previous report measuring pH in EBC (8) and a study that found that the temperature of collection is not a major determinant of EBC pH in healthy subjects (4).

In conclusion, our results suggest that the ECoScreen method can more efficiently collect EBC than the RTubeTM method and is more sensitive for mediators that are heat labile. Increased sensitivity is desirable when mediator levels are near the limits of detection, because higher levels allow greater precision of measurement in most assay systems. The main advantages of the RTubeTM are that it is easily transportable and less expensive, and is sensitive for some important characteristics of EBC including measurements of pH. Understanding the effects of the method on the efficiency of mediator recovery allows investigators to choose a method that will best meet the requirements of a given application.

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