Design and evaluation of a device for collecting exhaled breath condensate*

Diseño y evaluación de un equipo para obtener aire espirado condensado

Oscar Florencio Araneda Valenzuela, Maria Paulina Salazar Encina

Abstract

In recent years, the analysis of exhaled breath condensate samples has been given great weight as a noninvasive methodology of studying physiology and lung diseases. The present study describes a device for measuring exhaled breath condensate that is affordable, easily constructed, portable and suitable for use in the field, as well as allowing the collection of simultaneous samples. The results obtained with this device in terms of the concentrations of pH, hydrogen peroxide and nitrite, metabolites related to inflammatory and oxidative damage, in exhaled breath condensate samples are comparable to those obtained with other devices previously described.

Keywords: Exhalation; Lung diseases; Equipment design.

Resumen

El análisis de muestras de aire espirado condensado ha cobrado gran relevancia en los últimos años como método no invasivo de estudio de la fisiología y las enfermedades de origen pulmonar. En el presente trabajo se describe un equipo para tomar muestras de aire espirado condensado de bajo costo, fácil de fabricar, de transportar al terreno y que permite tomar muestras en forma simultánea. La concentración de metabolitos relativos a procesos inflamatorios y al daño oxidativo (pH, peróxido de hidrógeno y nitrito) de muestras de aire espirado condensado obtenido con este equipo son comparables a los reportados con otros previamente.

Descriptores: Espiración; Enfermedades pulmonares; Diseño de equipo.

In recent years, the analysis of exhaled breath condensate (EBC) has been proposed as a noninvasive methodology of studying lung diseases.[1] This tool has been used to search for mechanisms as well as to perform clinical follow-up of various pulmonary pathologies.[2,3] Exhaled breath is returned to the environment at a temperature of approximately 37°C, being saturated with water together with metabolism products and lung epithelial surface derivatives. Cooling of exhaled air by condensers captures part of this exhaled air through a system of hoses and tubes that offer little resistance to respiration. The product of this process is a transparent liquid containing volatile and nonvolatile particles, the concentrations of which are expressed using measures as small as micromoles per liter. Commercial devices have been developed for collecting EBC samples. Among such devices, the most widely used are the RTube™ (Respiratory Research Inc., Charlottesville, VA, USA) and the ECoScreen (Jaeger GmbH, Hoechberg, Germany). However, there are various models constructed by individual investigators for general use,[4] as well as for use in specific situations such as mechanical ventilation[5] or sample collection from suckling infants.[6] The particular interest of the investigators of the present study was to develop an EBC condenser that is affordable and portable, as well as allowing the collection of simultaneous samples, use in several experimental situations and use in patients with pathologies of pulmonary origin, either in hospitals or in the field, for the follow-up evaluation of exercise tolerance, as well as the effects of air contaminants, altitude and work.

Description of the device

Figure 1 presents a model of the device.

1) Connection to the condenser: Patients can be connected to the condenser via a mouthpiece or a mask. Although the mask is better tolerated by subjects, the mouthpiece allows the formation of approximately

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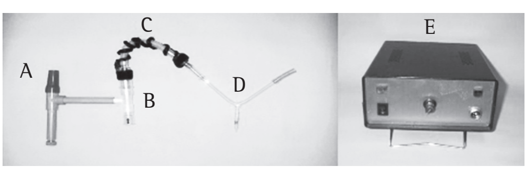
Financial support: none.
Submitted: 17 February 2008. Accepted, after review: 26 May 2008.

twice as much EBC in the same amount of time. For both forms of connection, a saliva trap should be used in order to avoid sample contamination.

2) One-way system: The one-way design allows the device to condense only the exhaled air and avoid contamination with substances in the environment. In our device, we adapted two valves, each measuring 22 mm in diameter (catalog numbers 1664 and 1665; Hudson RCI, Durham, NC, USA).
3) Flexible connector: The flexible connector allows subjects to move and adapt to a more comfortable position without interrupting or increasing resistance to exhalation. This can be constructed using a tube measuring 15 cm in length and 22 mm in internal diameter (catalog number 60-50-150-1; VBM Medizintechnik GmbH, Sulz, Germany).
4) Flexible heater: The device includes a mesh-covered electrical resistor whose ends are connected to a regulator that maintains the temperature at 37°C, preventing condensation in this area and increasing the collection yield.
5) Glass condenser: A Y-shaped glass condenser is used. The condenser has two upper arms, set at 45° angles, each measuring 120 mm in length and 8 mm in internal diameter. The flexible connector is attached to one end, and a hose, which allows the outflow of air, is connected to the other. At its lower end, the condenser has a third arm, measuring 40 mm in length, to which a plastic tube, which collects the sample, is inserted under pressure. In order to increase the EBC flow collected, more than one glass condenser, joined by flexible connectors, can be used.
6) Cooling system: A box containing crushed ice (−5°C), a mixture of ice and salt (−15°C) or ice packs (−15°C) can be used as a cooling system. The sample volume collected depends on the temperature and on the number of glass condensers used. As a reference, in an adult subject, 1.5 mL can be collected in 15 min when using crushed ice and a single condenser. Approximately twice as much is collected in the same amount of time when using two glass condensers and a mixture of ice and salt.

Note: The device was designed to be portable and to allow easy assembly/disassembly, as well as allowing the collection of simultaneous samples. When collecting samples from patients with pathologies of infectious origin, its parts can be discarded after use. However, the glass condenser can be sterilized and reused.



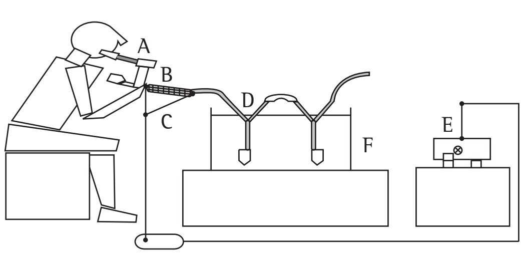


Figure 1 - Structure of the EBC condenser: (above) condensation system and temperature regulator; (below) general outline for assembling the device. Parts: (A) vertical mouthpiece and saliva trap; (B) one-way valves; (C) flexible tube and heater; (D) glass condenser; (E) temperature regulator; and (F) box containing ice.

Sample collection protocol

We recommend that subjects be comfortably seated, at rest and wearing a nose clip. Prior to sample collection, subjects should not eat for one hour and should not smoke for six hours. Due to the lower cost and the greater volume of condensate collected, we recommend that the mixture of ice and salt be used as the cooling method. Total time to collection under these conditions is 10 min or until subjects produce a sample of 1.5-2 mL (Table 1).

Chemical determinations

Various parameters, such as markers of inflammation, remodeling and tissue oxidative damage, have been determined in EBC samples.[2,3] Using the EBC condenser described in the present study, the concentrations of hydrogen peroxide, nitrite and pH were determined by different methods (Table 1), as were the concentrations of malondialdehyde,[7] 8-isoprostane and protein (data not shown). Although our condenser was not directly compared with others, the values revealed by the chemical analysis of the samples collected using the EBC condenser described here are at a level similar to previously reported values obtained using other condensers in healthy subjects (Table 1). In the future, this comparison should be carried out in order to allow a statistical analysis, which was not performed in the present report. Regarding the influence of the type of EBC condenser on the results, there have been reports in which other condensers were used indicating differences for parameters such as pH,[8-9] whereas others show no differences for aldehydes (malondialdehyde, hexanal, heptanal or nonanal).[10] Similarly, one group of authors,[11] using four different types of condensers, found no differences in condensate volume, nor in the concentration of hydrogen peroxide, 8-isoprostane or cytokine. This suggests that, in the search for standardization of EBC sample collection, it is necessary to standardize and describe the conditions under which samples are collected and handled, this probably being more important than is the type of condenser used. To date, there is no definite evidence that one condenser is more appropriate than others for collecting reproducible samples as suggested in the American Thoracic Society and the European Respiratory Society consensus on EBC collection methodology.[2]

In summary, the device described in the present study corresponds to a low cost system that has disposable parts and allows the collection of simultaneous samples from subjects under various environmental and experimental situations, providing results similar to those obtained with other EBC condensers previously described.

Acknowledgments

We wish to thank Mr. Luis Pizarro Zýñiga for his assistance in sample collection and chemical analysis. We would also like to thank Dr. Claus Behn for his comments and for facilitating the performance of the present study.

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Table 1 - Concentrations of hydrogen peroxide, nitrite and pH in the EBC samples collected with the exhaled air condenser described and literature values for samples collected with other condensers. The values found using the condenser described in the present study were obtained in samples collected from healthy, male nonsmokers (between 18 and 35 years of age), who were seated and at rest, using a mixture of ice and salt as the cooling system. Results are expressed as means ± SD and as median and interquartile range (25th-75th percentiles).

Parameter	Concentration obtained	Values in the literature	
H_2O_2 (μM)	0.53 ± 0.55	0.45 ± 0.29	Nowak et al. 2001[12]
	n = 26	n = 12	
NO_2^-(μM)	1.59 ± 1.00	0.55 (0.31 - 2.33)[a]	Hunt et al. 2001[13]
	n = 17	n = 20	
		1.8 ± 0.3	Nightingale et al. 1999[14]
		n = 10	
pH	7.69 ± 0.24	7.8 ± 0.6[b]	Paget-Brown et al. 2006[15]
	n = 37	n = 122	

H_2O_2: hydrogen peroxide; and NO_2^-: nitrite. [a]Parameters: The concentration of H_2O_2 was determined by spectrophotometry (FOX_2) with the addition of sorbitol, according to Gay & Gebicki 2002.[16] The concentration of pH was determined after aeration with argon, according to Paget-Brown et al. 2006.[15] The concentration of NO_2 was determined by the Griess reaction, according to Green et al. 1982.[17] [b]Corresponds to the group of patients between 21 and 30 years of age.

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