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.

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...
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 (−5ºC), a mixture of ice and salt (−15ºC) or ice packs (−15ºC) 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.

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. 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.

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References


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).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Concentration obtained</th>
<th>Values in the literature</th>
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<tbody>
<tr>
<td>$H_2O_2$ (µM)</td>
<td>0.53 ± 0.55, n = 26</td>
<td>0.45 ± 0.29, n = 12, Nowak et al. 2001&lt;sup&gt;[12]&lt;/sup&gt;</td>
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<tr>
<td>NO$\textsubscript{2}$ (µM)</td>
<td>1.59 ± 1.00, n = 17</td>
<td>0.55 (0.31 - 2.33)&lt;sup&gt;a&lt;/sup&gt;, 1.8 ± 0.3, n = 10, 1.8 ± 0.3, n = 10, Nightingale et al. 1999&lt;sup&gt;[14]&lt;/sup&gt;</td>
</tr>
<tr>
<td>pH</td>
<td>7.69 ± 0.24, n = 37</td>
<td>7.8 ± 0.6&lt;sup&gt;b&lt;/sup&gt;, n = 122, Paget-Brown et al. 2006&lt;sup&gt;[15]&lt;/sup&gt;</td>
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$H_2O_2$: hydrogen peroxide; and NO$\textsubscript{2}$: nitrite. *Parameters: The concentration of $H_2O_2$ was determined by spectrophotometry (FOX) with the addition of sorbitol, according to Gay & Gebicki 2002.<sup>[16]</sup> The concentration of pH was determined after aeration with argon, according to Paget-Brown et al. 2006.<sup>[14]</sup> The concentration of NO$\textsubscript{2}$ was determined by the Griess reaction, according to Green et al. 1982.<sup>[19]</sup> *Corresponds to the group of patients between 21 and 30 years of age.

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