



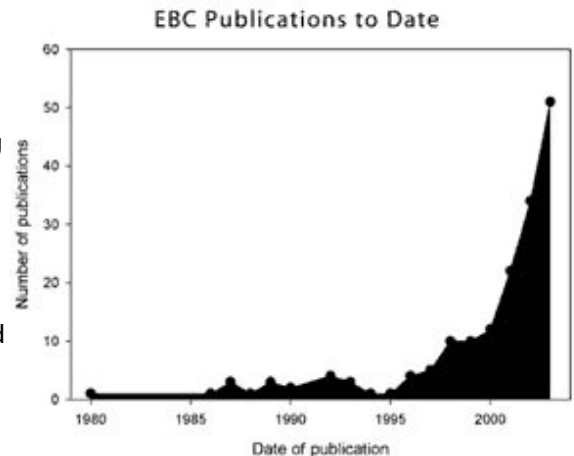
Exhaled Breath Condensate An Introduction

"A stuck tuning slide is one of the hazards of old flutes. The nasty organic compounds in your breath condensate continue working on the metals your slide is made from and effectively weld them together."

McGee's Flutes Home Page: www.mcgee-flutes.com

Flautists knew that there was much more than water, CO₂, N₂ and O₂ in our exhaled breath long before lung disease researchers figured it out. Probably so did every other wind instrument player: Clarinets, trumpets, Big French Horns. But they had something else wrong, however, for they thought that "there is no such thing as acidic breath" (www.musicchem.com). And we lung researchers trumped them on that one.

Exhaled breath condensate (EBC) was first reported as a human body fluid in 1980 (Sidorenko, Zborovskii et al. 1980) in the context of studies of surface active properties/surfactant. Since then, over 200 papers have been published, with most of these seeing press since 1995. There has been a rapid increase in publications in great part because of the strengths of the EBC method that are discussed below. But there are weaknesses as well, and they are likewise discussed below.



There are several review articles recent enough to provide timely background (Mutlu, Garey et al. 2001; Hunt 2002), and the American Thoracic Society and European Respiratory Society created a Joint Task Force on Exhaled Breath Condensate in 2001, which has finished its meetings and is currently revising its report for publication this year.

What is exhaled breath condensate?

Simply, EBC consists of 1) aerosolized particles of airway lining fluid evolved from the airway wall by turbulent airflow, that serves as seeds for substantial 2) water vapor condensation, which then serves to trap 3) water soluble volatile gases. The aerosolized particles contribute the non-volatile constituents of EBC, including ions and proteins. Dilution of these non-volatile constituents by the condensed water may amount to 3-4 logs. The water soluble volatiles are incorporated into EBC through entirely different mechanisms than the non-volatiles, and therefore dilution issue become essentially irrelevant. However what is relevant for the volatile components is their volatility and water-partition coefficients, which in part are inherent characteristics, and in part depend on temperature and pH of the source fluid. One important point worth reiterating is that EBC successfully samples both volatiles and non-volatiles, and they must be recognized as separate (although occasionally overlapping) entities with different properties.

Exhaled breath condensate contains every molecule that the airway lining fluid contains, but in very small concentrations. Thus, it contains ammonia, acetic acid, ascorbate, adenosine.....and all the way down the alphabet into the z's somewhere. Lists of identified substances can be found in various references (Hunt 2002) and also on our website.

What can EBC tell you?

Before that question can be answered, it is necessary for each investigator to reflect on what they are most interested in determining. Investigators fall into several categories.

First are researchers who wish to determine the components of the airway lining fluid environment in order to better understand disease, and who have recognized certain limitations of bronchoalveolar lavage (BAL) and induced sputum. These researchers wish to learn what the airway sodium concentration is, how many cysteinyl-leukotrienes are present in the airway in aspirin-sensitive asthma, and whether the airway lining fluid pH is alkaline or basic (to name a few of many questions). Interestingly,

these questions have not been successfully answered by any other method in healthy subjects, to say nothing of patients with disease. Researchers who wish to learn about actual airway concentrations of substances need to be aware that to date, there is no confident dilution marker for EBC (this remains true for BAL as well). However, as for BAL, ratios of the various non-volatile constituents can be determined, and deviations of these ratios compared with underlying pathologic condition. For example, IL-4 and IFN γ could be studied concurrently to identify an airway TH2 vs. TH1 profile (Shahid, Kharitonov et al. 2002). Products of leukotriene pathway compared to products of COX pathway can be compared. Ratios can also be determined for volatile acids and bases (such a ratio contributing to a measurable pH), and this can serve as information about the acidity of the source fluid (the airway lining fluid). In our minds, acidification of the airway is one of the key components of inflammation, and indeed incites inflammation. In other words, acidification comes first. The cytokines, chemokines and cells follow.

The second group are the clinical researchers looking to identify new enrollment criteria or outcome variables for their studies and are interested in identifying surrogate markers for lung disease. In like fashion to exhaled nitric oxide, biomarkers within EBC may be able to identify subjects with unrecognized lung inflammation, or patients whose lung inflammation is being sufficiently controlled. The key here is for the effect size to be sufficient to overcome dilutional concerns (for non-volatiles) and assay variability. For example, there may exist certain compounds in EBC that relate to underlying lung disease that are completely unmeasurable in health but prominently present in disease, and these sorts of markers may not require any dilution marker to be of marked interest. An example might be if an 80 kD Anthrax toxin can be identified in exposed subjects. Another might be that leukotriene elevations occur in asthma but much less so in health, and that groups can be classified based in part on their levels in exhaled breath. Conclusions require acceptance of the assumption that there is not a systematic difference in the dilution of EBC between asthma and health, however. (This assumption is not yet fully accepted.) This group of investigators is equally happy to have ratios serve as their biomarker of disease (such as cytokine ratios or pH)

A third group is interested in the EBC technique itself. These researchers want to understand the fluid they are collecting, develop dilution markers where necessary, maximize the collection of the relevant components of the fluid, develop more sensitive assays with minimal variability. This group consist of the technicians—the engineers.

The fourth group is the clinicians, eager to obtain the objective information necessary to make rational diagnoses and to titrate the various therapies for lung disease in individual patients. In this regard, it is quite fair to say that 1) our dosing of anti-inflammatory compounds is done essentially blind and 2) our diagnosis of acid-reflux induced respiratory symptoms is guesswork. If cardiologists were to prescribe cholesterol-lowering drugs without measuring cholesterol levels, they would get laughed out of the house and the insurance companies and governments would not pay for the drugs. Lung physicians prescribe inhaled steroids without any measure of lung inflammation. This glaring failure is desperate for a satisfactory solution. EBC holds promise to fulfill it.

How do you collect EBC?

Collection can be performed with simple home made condensation devices or with commercially available devices such as the RTube. All devices should be designed to as best as possible exclude gross salivary contamination (see caveats below). The materials used may be relevant for certain markers. For example, nitrogen oxides in EBC may be chemically reduced (for example to nitric oxide) by copper in metals. Or nitrogen oxides can increase from leaching of NO out of plastics (including Teflon and many others), latex, and other materials. Very large condensate collectors have even been built for horses. Collections of EBC have been performed at altitude, in environmental exposure units, in intensive care units, schools, homes, clinics and worksites.

Sampling is generally accomplished by having patients breath at tidal volumes orally into a mouthpiece attached to a cold condenser. There are multiple variations, but all on that theme. EBC can be collected through nasal cannula (Griese, Noss et al. 2002) as well as through endotracheal tubes (Gessner, Hammerschmidt et al. 2003; Vaughan, Ngamtrakulpanit et al. 2003; Moloney, Mumby et al. 2004). Collection times can be as short as 90 seconds (Vaughan, Ngamtrakulpanit et al. 2003), but some investigators require over an hour to obtain sufficient EBC. Ten minutes of breathing is commonly employed. Most studies reported to date involve spontaneously breathing patients sitting comfortably.

A list of Caveats

1. EBC is an evolving field. Beware of dogmatic preachers. Nobody has a full understanding of this material, and if they claim they do, they are overconfident.
2. There is no dilution marker yet, although it will be easier to find than for BAL, we believe.

3. Differentiate in your mind the volatile constituents from the non-volatiles.
4. Beware of assay variability. Many markers are detected in low concentrations—often at the lower limits of the relevant assays. Intrasample assay variability can be, and often is, the principal contributor to EBC variability. In other words, the sample collection procedure and storage may reproducibly collect airway lining fluid particles and volatiles, but the assay employed may suffer from too much non-specific background noise to be able to accurately measure the constituents. Higher sensitivity assays with better noise suppression are one solution. Seeking large effect-size biomarkers (substances which differ more substantially between health and disease than the assay noise, either because noise is low, difference in disease is high, or both) is another solution.
5. Standardization issues. There are as yet no standards available for EBC collection, storage and processing. Each biomarker studied will have separate issues that need to be addressed— different stabilities, different reactivities, different volatility—and therefore global standardization for all biomarkers is not a reasonable or rational goal. Instead, standardization for collection, storage and assay for each individual biomarker of interest needs to be determined based on empiric data collection. For now, journal readers are best served if publications regarding specific markers in EBC fully describe the methods employed, and discuss intrasample and intrasubject assay variability. Efforts at developing a fully standardized method (a one-stop shop) are unlikely to be successful, anymore than treatment of asthma can all be done one way.
6. Oral contribution. In the context of EBC, the “airway” may include everything from the alveoli to the back of the teeth. Oral collections of EBC assuredly incorporate some amount of oral influence. The issue is whether this oral contribution is relevant. Using amylase activity assays, it is rare to find any identifiable salivary amylase in EBC, which means that if it is present at all, saliva is diluted at least 10,000 fold in EBC. However, amylase is likely identifiable with more sensitive assays. Additionally, amylase can be identified in isolated lower airway samples and BAL, and may indeed be excreted in the lung itself, thus confusing the matter further. Absence of identifiable Phosphorous has been suggested to be supportive of absence of saliva (Griese, Noss et al. 2002). Many papers study EBC in intubated subjects, thus reasonably well, but not completely, avoiding oral contamination of EBC (some saliva may accompany the endotracheal tube on its trek through the cords). Indeed, in general, more protein (not amylase) is recovered from endotracheal collections than from oral collections (Gessner, Hammerschmidt et al. 2003). This raises an interesting possibility: particles larger than 5 microns that are inhaled orally tend to be trapped by impaction on the retropharynx and cords. It is reasonable to consider that particles generated in the lower airway that are larger than 5 microns might likewise be trapped during exhalation by impaction as they round the bend of the pharynx, but that the endotracheal tube may decrease that effect by decreasing the turbulence of the exhalation, allowing more (and importantly larger) particles to be collected.
7. Nitrogen oxides. These compounds are everywhere, and contaminate lab surfaces. Nitric oxide gas diffuses through many materials—evolves out of others—and becomes oxidized to nitrite and nitrate. Thus these higher oxides of nitrogen, in addition to coming from the lung (both in particle form and from oxidized exhaled nitric oxide), are found in pipettes, microcentrifuge tubes, on latex gloves, and just about anything else that comes in contact with EBC, unless stringent efforts are undertaken to minimize contamination.

(See also: Methods to Avoid NOx Contamination)

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