The collection of Exhaled Breath Condensate

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Composition of Exhaled Breath

- Exhaled Breath (~37 °C) is mainly composed by gaseous substances: Water Vapor, CO_2, O_2, N_2, NO, CO, etc.

- Several volatile, low-volatile and non-volatile compounds of endogenous and exogenous origin are present in traces:

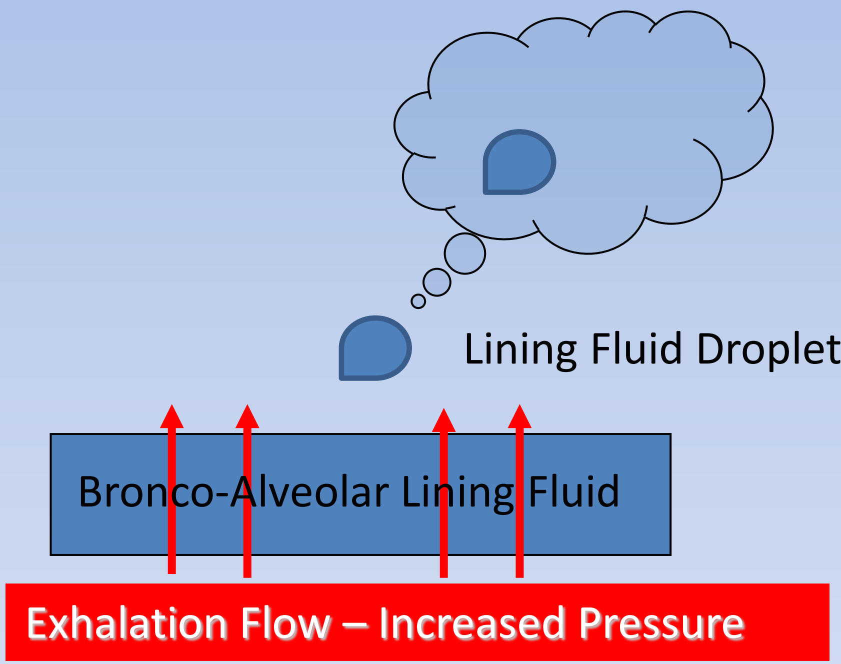
Volatile: VOCs, linear aldehydes, ammonia, free radicals, etc.

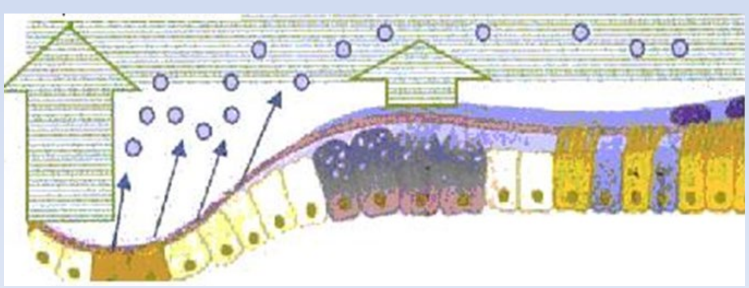
Low-Volatile: products of peroxidation, H_2O_2, other aldehydes, fat acids, lipids, etc.

Non-Volatile: metallic ions, non-metallic ions, proteins, DNA, cellular membranes, etc

Exhaled Breath Composition may be representative of airways status and is altered in several pulmonary pathologies.

Why non-volatile substances in exhaled breath?



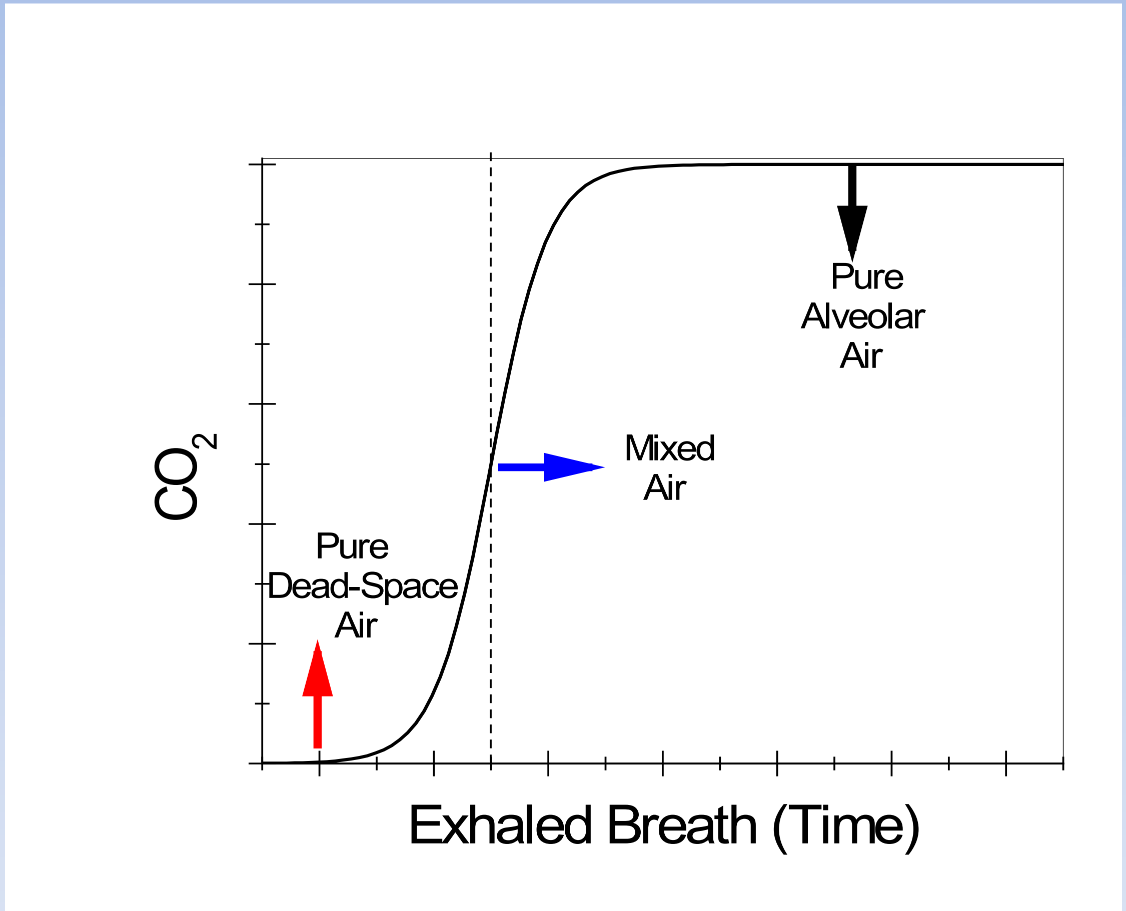


Pressure, very small size (<10 micron) and high charge make the droplets a vapor, although they contain non-volatile substances

Exhaled Breath is a BioAerosol

Suspension of non gaseous small particles or droplets that contain substances released from living organisms (e.g. airway cells).

Phases of Exhaled Air



Exhaled Breath can be divided in three phases following the Fowler's graphical method for CO2 =>

This curve can change on the basis of (1) the chemical and physical properties of the considered biomarker; (2) its endogenous/exogenous nature; (3) its airway origin and deposition (upper and lower airways).

Exhaled Air Condensate (EBC)

Exhaled Breath Condensate is obtained by cooling the Exhaled Breath BioAerosol at low temperature (-50=>5 °C depending by the collection device.

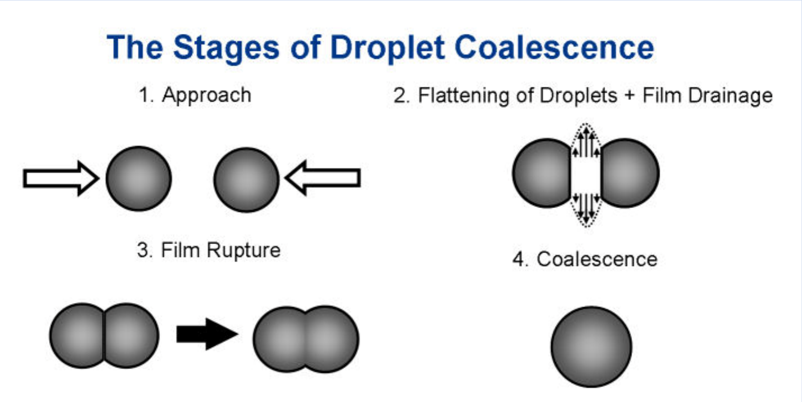
EBC is mainly composed by water, but contains also ions deriving from Exhaled air gases (carbonate, ammonium, etc), non- or low-volatile compounds and high volatile compounds on the basis of collection temperature.

Mechanisms of formation of EBC

EBC is formed by CONDENSATION of water vapor and other substances present in exhaled air at low temperatures. Henry's law determines the concentration of them inside EBC.

Other phenomena concur, with an important influence on the collection of lining-fluid droplets:
NUCLEATION (Cloud Condensation Nuclei) through COALESCENCE:
the process by which two or more droplets, bubbles or particles merge during contact to form a single daughter droplet, bubble or particle.

Raindrop: As small water droplets are carried by the updrafts and downdrafts in a cloud, they collide and coalesce to form larger droplets. When the droplets become too large to be sustained on the air currents, they begin to fall as rain.



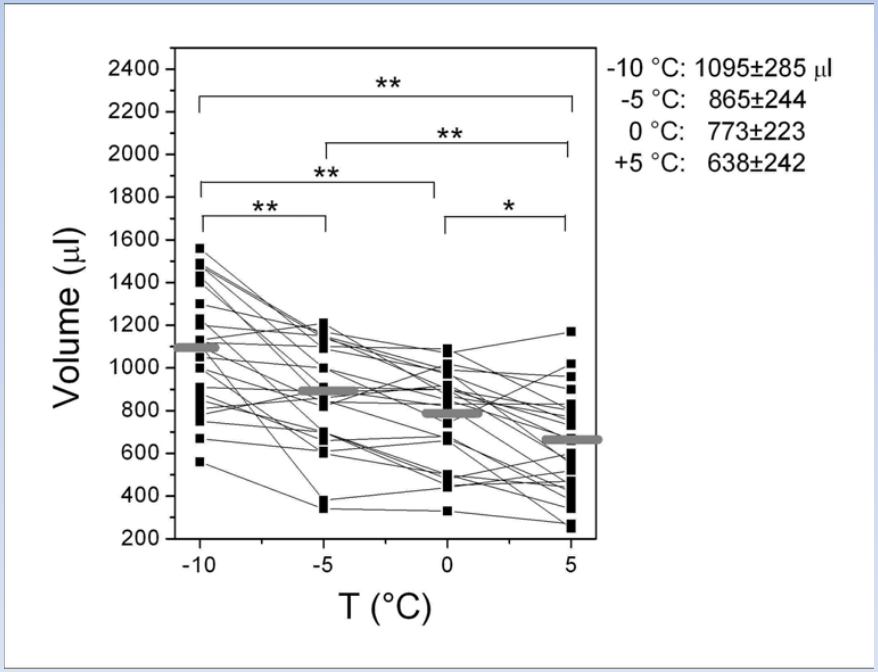
A cold solid surface favors coalescence => The yield of collection in EBC of lining-fluid droplets should be very high below 0 °C.

Henry's Constant

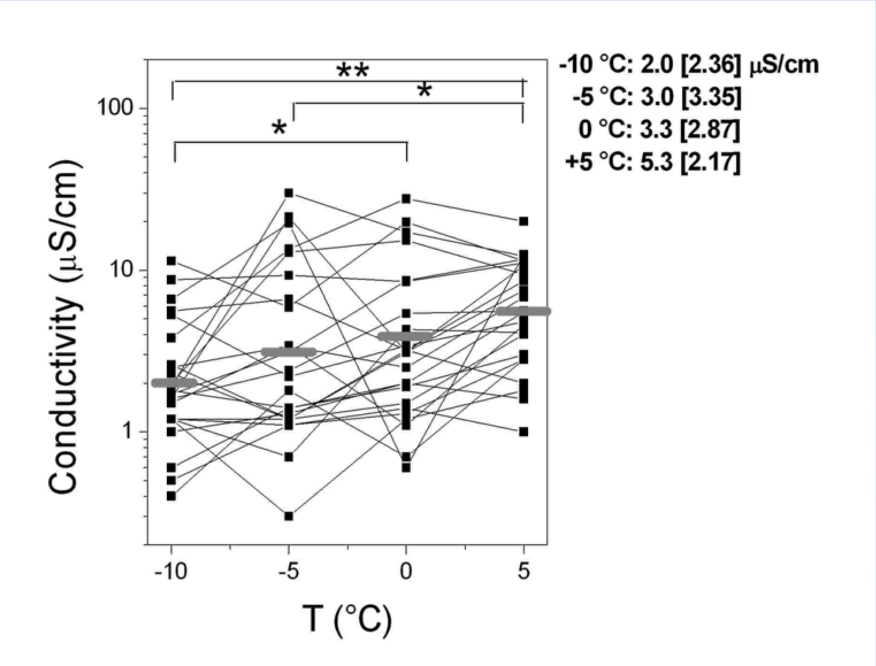
Compound	Henry's Constant in water at 25 °C (mol/Kg*bar)
O_2	0.0013
NO	0.0019
CO_2	0.034
Benzene	0.18-0.21
Nonanal	1.0
Heptanal	3.3-3.7
Hexanal	4.7-4.9
HCl	19
Acetone	25-27
NH_3	27
OH°	30
2,3-Butanedione	57-74
Ethanol	200
HO_2°	4000-9000
H_2O_2 (25 °C)- H_2O_2 (37 °C) dilute solutions	67500-23700
Glycerol	10^{11}
Non volatile Ions- Proteins-DNA	->INFINITE

Mechanisms of formation of EBC

Due to Nucleation, Decreasing condensation temperatures DO NOT increase the yield of condensation of lining fluid droplets and non volatile substances =>
From a physical point of view, the dilution factor of non volatile substances IS INCREASING when the Collection temperature IS DECREASING.



Water collection Efficiency



Dilution of non-volatiles

The Collection Temperature

An excessive decrease in collection temperature (<-10 °C) increases the volume of EBC, but induces a dilution of low-volatile and non-volatile molecules.

<u>NB: almost all biomarkers measured in EBC are in this category.</u>

We suggest to measure the molecules more volatile than the water directly in exhaled breath by means of GC-MS or other techniques.

An excessive high collection temperature (> +5 °C) can excessively reduce the volume of EBC, with consequences on the number of biomarkers measurable in it (e.g. few aliquots).

Collection Temperature Should be chosen on the basis of the stability of the selected compound.

A compromise of 0 °C / -10 °C seems acceptable for almost all biomarkers currently in use.

EBC contaminations

- SALIVA: Collection Device should be equipped with a saliva trap: only vapor phase should reach the collection tube and the condenser.

- AIR CONTAMINATION (polluted environments): environmental air should not reach the collection tube during the collection in polluted environment.

- All the collection system should be DISPOSABLE and should neither release compounds in it (the risk of glass, metal, and some plastics) nor entrap some biological molecules (e.g. some proteins) => inhert. Highly critical for metallic and non-metallic elements.

- EBC should remain in only a phase, possibly the liquid phase. Partially liquid/iced samples and repeated defrosting can damage some molecules (e.g. highly reactive compounds and interleukins).

The conditions of best collection

- The device system should not have crosses or high constrictions => condensation could start outside the collecting tube, with a loss of non-volatile molecules.
- Collection inside the tube is favored when the exhalation flow has a relevant vertical component in the direction of gravity force.
- Collection device should be portable, light and easy to handle.
- Collection should be normalized for an external parameter: (a) time of collection (e.g. all the subjects collect their ebc for a standard time); (b) exhaled air volume (e.g. all the subjects exhale the same volume of air independently by time).
- Collection should permit the study of the chemical and physical properties of the molecules dissolved in it (see the next slides).
- The resistance of the circuit could be low. All the subjects should collect their EBC without any effort.
- Nose clip should be optional, and the collection device should permit internal respiratory cycles (e.g. subject does not need to leave it to inhale).

Our routinely Collection

The subjects are asked to breath tidally through the mouthpiece without a nose clip for 10/15 minutes at collecting temperatures of -10°C-0°C.

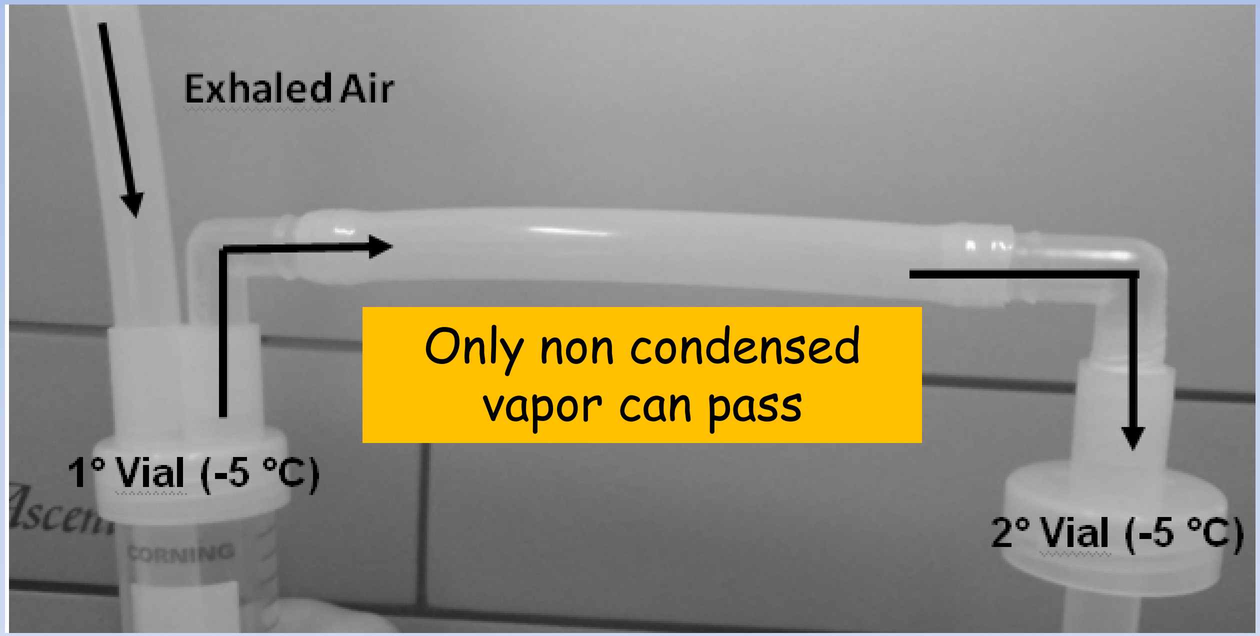
The subjects can collect their EBC at our laboratory/home/workplace and can perform repeated collections.

The subjects are strictly instructed to maintain constant tidal breathing during the test and to form a complete seal around the mouthpiece; excess saliva IS periodically eliminated and the mouth was rinsed with water (indicatively every 5 minutes).

The EBC samples are centrifuged for 1 min at 1000 g immediately after collection so that all of the water droplets are driven to the bottom of the flask, and the total volumes are measured.

The samples are stored at -20 °C/-80°C until analysis.

Condensers in Series (Corradi et al. 2008)



Traditional Collection

Secondary Collection

Condensers in Series (Corradi et al. 2008)

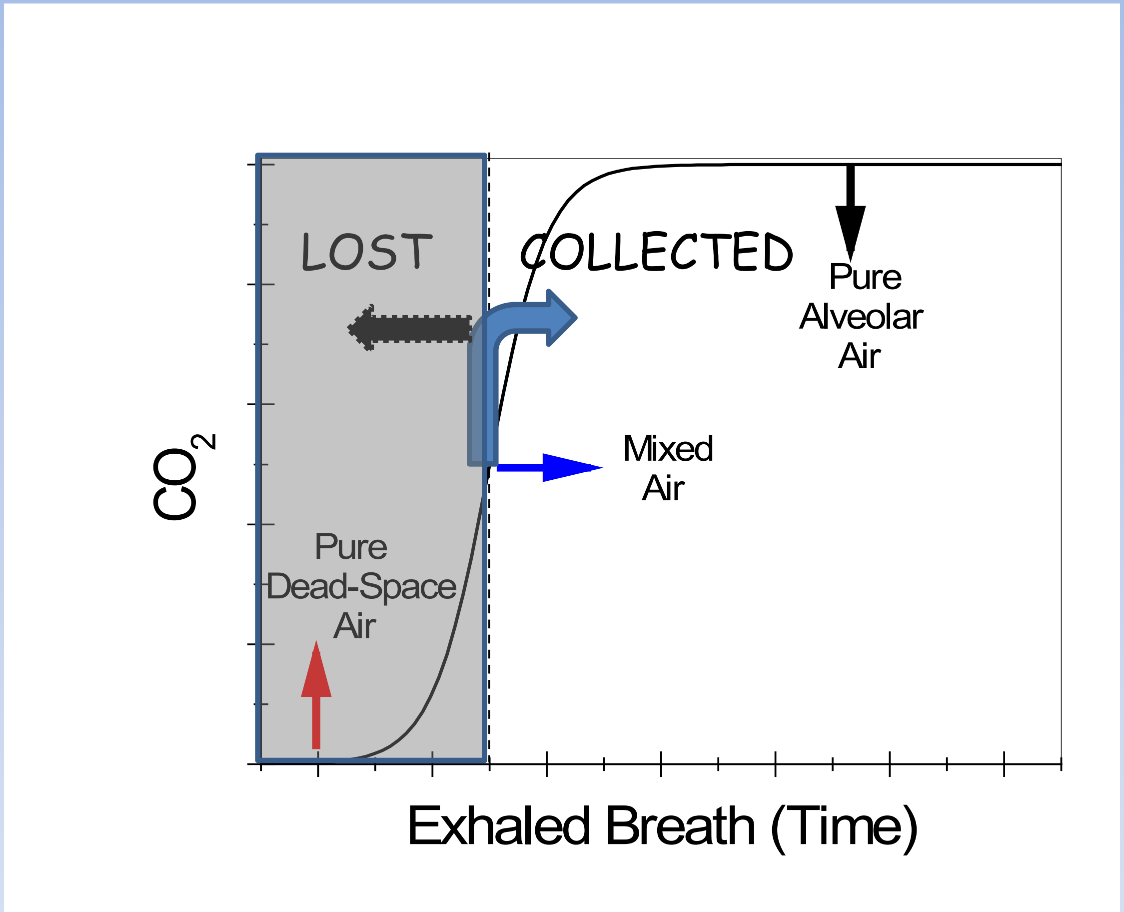
	Non-smokers	Smokers
Conductivity (EBC1) µS/cm	54 (32-93)	29 (19-36)
Conductivity (EBC2) µS/cm	36 (17-65)	20 (16-30)
Lyoph. conductivity (EBC1) µS/cm	1.9 (1.3-2.1)	1.3 (0.9-2.0)
Lyoph. conductivity (EBC2) µS/cm	ND (ND-0.7)	ND (ND-0.7)

Low Decrease

High Decrease

Ions deriving from gases can form in both aliquots, while non-volatile ions cannot pass in the second vial. => 15% max

In Development



EBC arises from all the exhaled air =>
A valve which permits the condensation of prevalently alveolar air can avoid the contamination due to the Dead-Space air and limit the contribution of upper airways.

A kit to measure pH directly in EBC (acidification of the airways) without any need of a laboratory is currently available.