



## Deaeration for EBC pH Assay — How is it Performed?

Measurement of Exhaled Breath Condensate pH is obviously a very simple assay to perform. There are some tried and true techniques that may be of use to you, and we will include these below. Deaeration is a very simple process that may help you with your study design and assays.

Please see “Why Measure EBC pH” for reasons why you should strongly consider measuring the pH of the EBC.

Deaeration is a simple process that standardises EBC CO<sub>2</sub> levels. CO<sub>2</sub> is a precursor of carbonic acid, which can acidify EBC somewhat, but which tells us nothing about the airway lining fluid pH. Controlling for CO<sub>2</sub> allows us to see in EBC the effects of other acids—ones that are volatile from acidic source fluids.

Deaeration involves bringing an EBC sample into equilibrium with a carbon dioxide-free gas. We have, now traditionally, used Argon gas as our deaeration gas, and Argon of course readily removes oxygen and other dissolved gases from EBC as well. If one were to use room air that had been filtered to remove CO<sub>2</sub> from it, then the process would be more reasonably considered decarbonation (the opposite of soft-drink carbonation—the terms are not satisfactory however as carbon is not the substance we are addressing). Nitrogen can also readily be used for deaeration.

We have used Argon because it is:

1. inexpensive
2. non-toxic (see important caveat below\*)
3. heavier than air. Being heavier than air, Argon effectively sits atop the sample during and even for a time after deaeration, preventing and/or delaying the opportunity for ambient CO<sub>2</sub> to recontaminate the sample and artificially lower the pH.

As a noble gas, Argon will not react chemically with tissue, BUT it can of course be dangerous to inhale in concentrated form. This is because of the very fact that it is heavier than air, and if inhaled will displace oxygen from the alveoli. It can be difficult to get the Argon back out of the alveoli and hypoxia can develop. Thus if large amounts of Argon gas are to be used (deaerating dozens of samples) we recommend doing so under an exhaust hood. In any event, assure that the room is well ventilated.

Deaeration involves bubbling Argon (or another CO<sub>2</sub>-free gas) through the sample until the pH stabilizes. During the several minutes of deaeration, the pH of the EBC can be seen to steadily rise (see caveat below) as CO<sub>2</sub>—and carbonic acid—are evolved from the EBC. When the pH stops rising (stabilises), the pH is then recorded. This is at the point that all CO<sub>2</sub> that can be removed by deaeration has been removed.

The bubbling process is straightforward. It can be performed by placing a pipette into the sample and running Argon through the pipette at an appropriate flow. The key is to assure that there is constant exposure of the Argon to the sample in a manner that is constantly breaking the sample surface tension, so as to maximize the ability of the CO<sub>2</sub> to be released. This is seen when the sample is constantly roiling/bubbling. It is possible to run the Argon flow too rapidly, especially in a small sample, and this can lead to the sample being pushed out of the way by the Argon while surface tension holds the sample intact, suspended by the flow on the wall of the container. This is suboptimal.

For a 300 microliter sample, 250 ml/min of Argon flowing through a glass pipette for 10 minutes is sufficient for EBC samples to be deaerated to completion. You can monitor the sample's pH during deaeration if you choose, or just measure after 10 minutes. Of course we recommend that you perform your own controls so that you are comfortable with the assay.

We are developing other methods to deaerate which take as little as 1 minute, and hope to make those methods available in the near future.

Pearls:

1. The sample can be deaerated any time.
2. The sample does not need to be plunged immediately after collection when pH is of interest. It can be stored indefinitely

- in a freezer if both ends are capped. If only the upper end of the RTube is capped, it is wise to plunge within a week.
3. The sample can be reassayed even years later just by deaerating again.
  4. Sample freeze-thaw does not affect EBC pH.

Argon gas can be ordered from any medical gas supply company, and is very inexpensive.