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Deaeration for EBC pH Assay — Why?

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 consider measuring the pH of the EBC.

Is it okay to not deaerate EBC before measuring pH? (see: "Do You Need to Dearate EBC Before Measuring pH?")

Let us first ask why one might not want to deaerate.

1. We frequently hear that people do not want to deaerate their samples because they wish to include CO_2 in the assay because it is a relevant acid in the airway, and removing it would in their minds make the pH result misleading. However, we believe that leaving the CO_2 in EBC is more misleading. This is because the airway lining fluid itself has a mild buffer capacity that can absorb CO_2 without changing pH very much. However, EBC is much less buffered, therefore the partial pressure of CO_2 found in the airway will lead to a more pronounced EBC pH change than it would cause in the airway lining fluid itself. Thus EBC pH, when not deaerated, is artificially lower than airway lining fluid pH. It therefore can be misleading to leave the CO_2 as a factor in the EBC.
2. Expense and effort. We use Argon gas, which is extremely inexpensive and readily obtainable. Effort involved is relatively small, and is necessary to increase the flexibility of our study designs. Please see below.
3. Some people have read the speculations that deaeration can lead to EBC acidification through effects of oral ammonia and asthmatic hyperventilation. These arguments have always been wrong on both theoretical and empiric grounds. (see: "What About Oral Ammonia?", and "Can Hyperventilation Affect EBC pH?")

Why would I want to deaerate EBC before measuring pH?

1. Carbon dioxide dissolves in EBC to partly form carbonic acid, which releases protons and acidifies the fluid. We have good ways of measuring exhaled CO_2, however, and do not need to use EBC for this purpose. What we are more curious about is the production of other acids from the airway-specifically acids that are, unlike CO_2, only volatile when the pH of the source fluid is low. These are the acids that can tell us something, whereas exhaled CO_2 tells us nothing about the pH of the source fluid, yet can contaminate the EBC pH reading. Thus we generally recommend standardising the amount of CO_2 in the sample in some fashion, and deaeration is one easy way to do that.
2. Deaeration of EBC provides for pH readings that are very similar to pH readings of tracheobronchial mucous. Mean EBC pH readings are in the 7.7 to 8.0 range, the same range as found in tracheobronchial mucous(1). A caveat is that tracheobronchial mucous is only one "compartment" of airway lining fluid.
3. Incorporation of deaeration into the assay protocol allows for a much simpler study design. There is no need to measure pH right away. Subjects can and have collected samples in their own homes, stored samples in their home freezers for weeks, and brought them into the lab at mutually convenient times. This is an example of just how flexible the assay can be. Another example might be using the RTube to collect sample from 100 workers at a time in a coalmine or swine-containment facility. One hundred samples can be collected within a total of 20 minutes, allowing the workers to get back to their jobs, while the assays can be performed any time thereafter.
4. Deaearated pH measurement is the most extensively validated assay in the EBC field(2). Potential technical confounders have been examined in detail. EBC pH is not affected by any of the following factors:
    1. Duration of the collection
    2. Volume of sample collected
    3. Hyper- or hypoventilation of the patient
    4. Time of day collected
    5. Presence or absence of an expiratory particle size filter
    6. Storage temperature of the sample

7. Time between collection and assay

We do believe it is important when considering pH to use a condenser temperature that is not too cold. By this, we mean that it is necessary to avoid condensing EBC in the form of ice/snow, but instead we should condense in liquid form. Condensing in the form of snow/ice may prevent the absorption into the EBC of the volatile acids in which we are interested. Chilling the RTube aluminum condenser in a standard home freezer (-4 to -15° C) is completely satisfactory (and entirely convenient too!), as then the EBC condenses on the RTube collector wall without freezing.

There are only a few issues for which to control: subjects should not eat or drink anything that might contain volatile acids (particularly vinegar) within 1-2 hours of providing EBC sample. Foods of concern include vinegar itself, apple juice (which contains vinegar if it is not perfectly fresh), potato salad, tomato catsup (ketchup, again containing vinegar) and pickles. Most subjects can have these ingestions and within 20 minutes have resolved the effect on their EBC pH. However, a small subgroup (presumably with low buffer capacity of their saliva) will show persistent EBC pH changes for 1 hour, and occasionally for as much as 2 hours after ingestion. This confounder is simply an example of a small portion of the airway becoming acidic and releasing volatile acids that can affect EBC pH.

Alcohol ingestion sufficient to cause inebriation has led to mild EBC pH effects many hours after ingestion. This is presumed (but not assuredly) caused by production of acetic acid from alcohol metabolism. Recently, the enzymes involved in this pathway have been identified in the lung.

Additionally, we have some preliminary data to suggest that EBC pH can decline in association with physical and emotional stress. We don't interpret this to be a confounder, but rather that stress may lead to airway acidification.

References

1 Metheny, N. A., B. J. Stewart, L. Smith, H. Yan, M. Diebold, and R. E. Clouse. 1999. pH and concentration of bilirubin in feeding tube aspirates as predictors of tube placement. Nurs Res 48(4):189-97.

2 Vaughan, J., L. Ngamtrakulpanit, T. N. Pajewski, R. Turner, T. A. Nguyen, A. Smith, P. Urban, S. Hom, B. Gaston, and J. Hunt. 2003. Exhaled Breath Condensate pH is a robust and reproducible assay of airway acidity. Eur Respir J 22(6):889-94.