



## Why Measure EBC pH?

To answer this question requires consideration of how inflammation damages the airways and lungs. Inflammatory cells do not cause host damage just by sitting around, nor by releasing cytokines or other mediators.

Inflammation damages the host in many of the same ways that it was designed to damage invading microorganisms. These mechanisms principally include:

1. Release of enzymes that allow access of phagocytic cells to subepithelial microorganisms. The classic example is neutrophil elastase, which drills holes in tissue to allow tissue neutrophil migration to get the site where those bacteria are.
2. Release of proteins toxic to organisms that also have toxicity to human tissue: eosinophil proteins such as Major Basic Protein and Eosinophil Cationic Protein come to mind. Probably designed to assist in burrowing through the surface of a worm, these proteins aren't too intelligent and will do the same to the surface of the airway.
3. Release of oxidants, such as derived from neutrophil oxidative burst (and many other pathways). Oxidants essentially burn the target. Damage from oxidants will occur to proteins, lipids, and nucleic acids, and therefore can greatly affect the function of enzymes, messengers, membranes etc, in a slower, but nonetheless similar manner that a burn injury effects the function of the skin. Skin burns are usually painful. Pain in the airway is sensed as a desire to cough.
4. Release of acids. Most inflamed tissue is acidic. Many microorganisms do not survive well in acidic fluid, therefore controlled acidification of tissue may serve an important innate immunologic role. Acid is released by airway epithelium almost immediately upon contact with organic dusts and humans start exhaling large amounts of volatile acids within hours of infection with rhinovirus (common cold). This exhalation of acids (acidopnea) occurs 24-36 hours before any symptoms of a cold appear, and before any other physiologic, biochemical, or immunologic disturbance can be identified.

There are multiple toxicities of an excessively acidified airway, and these toxicities reflect the pathologic process of asthma. Acid causes the following:

1. Cough. Protons (acid) are potent triggers of the capsaicin receptor which then leads to vagal-mediated cough, bronchoconstriction, and glandular secretion, as well as local axonal release of pro-inflammatory tachykinins. The capsaicin receptor is a vanilloid receptor that we now commonly refer to as the "acid receptor" given the likely evolutionary influence toward expelling acid from the lungs. Indeed, it is not likely that mammals evolved to expel jalapeno peppers! More likely, the jalapeno pepper evolved the capsaicin to prevent being ingested by mammals!
2. Epithelial dysfunction and sloughing. In vitro, epithelial surfaces slough off when incubated at mildly acidic pH values. In animal airways, bathing the tissue at a pH of 6.2 leads to epithelial sloughing. During asthma exacerbations in humans, the airway epithelium sloughs, exposing the underlying tissue, removing a key immune barrier, promoting fluid exudation, and eliminating a source of important epithelial-derived physiologic modulators.
3. Eosinophil inflammation. Eosinophils cannot undergo anti-inflammatory apoptosis when the conditions are mildly acidic. By default, then, these cells necrose, and in the process release a variety of proteins and oxidants that are damaging to host tissue.
4. Mucous plugging. Respiratory mucous converts from the liquid sol phase into the viscous gel phase below a pH of approximately 6.5. This gelatinous mucous does not flow well, and can get lodged in airways.
5. Surfactant is made dysfunctional when acidified. With surfactant being critical not only for alveolar stability, but also for small airway patency, dysfunctional surfactant may be an important part of the quickly reversible air trapping identified in asthmatics studied with hyperpolarized Helium3 magnetic resonance imaging.
6. The airway redox systems, which incorporates the anti-oxidants, are prominently affected by the pH of the local environment. In general, acidification enhances oxidant stress.

Acid stress in the airways not only complements oxidant and inflammatory stresses, but contributes to them. Indeed, airway acidification in theory underlies a great many of the pathologic processes that occur in asthma and other respiratory diseases. Therefore it has not been surprising that airway acidification, as determined by increases exhalation of acids trapped in EBC, has been identified in asthma, COPD, cystic fibrosis, Acute Respiratory Distress Syndrome, Acute Lung Injury, and after pulmonary

resection. This acidification has been found both in oral collections and in endotracheal collections in which no oral contribution was possible. Importantly, EBC acidification occurs within hours of nasal infection with rhinovirus. As rhinovirus (the common cold) is an extremely important cause of exacerbations of chronic respiratory diseases, one can quickly suspect that the mechanism of triggering exacerbations lies in the human airway acidification response to rhinovirus exposure.

Gastroesophageal reflux to the level of the larynx and into the trachea is an important contributor to EBC acidification in some subjects, such as those with acid-reflux induced cough (with or without underlying lung disease). There may be particular value to this finding in studies of proton-pump inhibitor efficacy in respiratory diseases. The more a subject has EBC acidification in close temporal association with cough symptoms, (in the context of normal pH when not coughing), the more likely it will be that the cough is acid-reflux related. It remains unclear the extent to which gastroesophageal reflux contributes to EBC acidification. Indeed it may well be an important contributor, although it is unlikely to be dominant. For example there is no reason to suspect that GER increases 2-4 hours after nasal rhinovirus inoculation, before any cold symptoms appear, and yet the EBC become acidic.

In addition to the central pathologic relevance of airway acid-base balance, there is another reason to study EBC pH. It is the most validated of all the EBC biomarkers. EBC pH assays (performed after simple deaeration with Argon) are extremely robust. There is no dependence of the pH on duration of EBC collection, volume of EBC collected, patient ventilation levels or effort, site of collection (oral vs. endotracheal), patient age or sex. There is essentially no diurnal or daily variability in healthy subjects (although ingestion of food or drink with volatile acids (vinegar) can affect the assay for as much as two hours thereafter). Measuring pH is an extremely sensitive assay, and very inexpensive. Importantly, the effect size of pH decline seen in diseases is overwhelmingly greater than any assay variability. (see: "Exhaled Nitric Oxide")

In summary, EBC pH is an indicator of airway acidification. Airway acidification is a key recent finding that explains much of asthma pathology (as well as other airway diseases). Therefore the assay is particularly relevant to our understanding of lung and airway diseases, and may be particularly useful for understanding acid-reflux associated respiratory symptoms. The pH assay is simple, inexpensive, and immune to technical confounders. The effect size is large enough to assure valid and interpretable data as an outcome variable in studies. The measurement of EBC pH is, in our opinion, the single most comprehensive and useful measurement made in the exhaled breath.