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What pH Probe Should I Use?

This is an important issue. EBC is considered a very dilute fluid, although far from being pure water. Nonetheless, it is wise to use pH probes that are designed for aqueous solutions with relatively low dissolved material. Many glass electrodes are specifically designed for use with ultra pure water and are sold as such, although many other electrodes not so specifically labeled serve quite well in EBC.

Glass electrodes are quite usually effective. In EBC, as in pure water, a slight leak of the electrode's filling fluid (often KCl K⁺) is desirable to have (and a common characteristic of probes used in relatively pure water). This leak assists in more rapidly stabilizing the pH upon inserting the probe.

It is wise to calibrate your pH probes in appropriate calibration buffers frequently. As a general guide, pH calibration should be performed every 20 assays, and within an hour before any sample measurement. Consider testing your probe with a low osmolarity calibration buffer as well. These special calibration buffers are designed to assist with very precise pH measurement in very pure water. In this regard, the ionic strength of a solution does affect the measured pH, although the effect in EBC is small enough to be ignored completely.

Temperature has well known effects on pH measurement as well. Again, within the range of temperatures we are dealing with (between 0 and 37° C) these effects in EBC are small. During deaeration, the temperature of the EBC is usually approximately 14-17° C in our laboratory. If measured at near 0° or near room temperature, the measured pH might be 0.2 units different (depending on the probe used). **The effect size of the pH (the changes seen with disease) are much greater than the small effects of temperature or ionic strength on the pH measurement.**

Stabilization of the pH reading

Aspects that lead to more rapid measurement stabilization are to measure as small a sample volume as possible and use an electrode that is designed for purer water assays.

This will allow for the probe and meter to most rapidly stabilize and provide the actual pH. If a once rapidly stabilizing pH probe later stabilizes slowly or poorly, the H⁺ permeable membrane or reference has likely become clogged with proteins. Follow the instructions for cleaning the electrode that came with it, and often the probe can be resuscitated.

Stabilizing the pH itself

pH in EBC will change as CO₂ moves in and out of it. For example, exhaling over the top of the EBC will decrease the pH transiently, and of course EBC left out standing in room air after collection will show a gradual pH rise as CO₂ is evolved. These issues are eliminated if you employ deaeration before pH measurement—a procedure we generally encourage. (see: "Deaeration for EBC pH Assay — How is it Performed?" and "Deaeration for EBC pH Assay — Why?" for discussions of deaeration)

Over the years we have used several different pH probes. The probe that we use currently is a microelectrode manufactured by Orion.

Specific and recent information can be obtained by sending an email to info@respiratoryresearch.com with your questions.