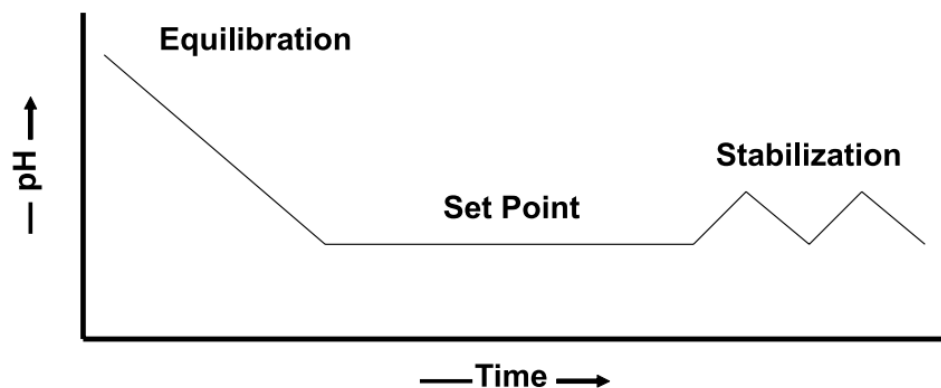


pH monitoring in embryo culture conditions

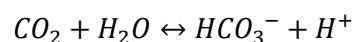
Many of us have heard about how important pH is for embryo culture. For example: denuded mature oocytes lack robust mechanisms to regulate internal pH, cryopreserved/warmed embryos have a reduced ability to regulate internal pH for several hours, and cleavage stage embryos have reduced ability to regulate internal pH compared to post-compaction embryos. Importantly, changes in intercellular pH of embryos impacts metabolic activity, can impact organelle localization, and can even influence resulting fetal development. Thus, maintaining a stable and appropriate external pH (media pH) is critical.

Media pH is dynamic and changes during embryo culture.



Swain, JE. (2010) "Back to Basics: pH for the ARTisan." *Journal of Clinical Embryology*. 13(2):9-28.

When medium is used it must first be equilibrated to obtain the proper growth environment. Placing the medium in the incubator overnight allows enough time for the media to absorb CO_2 and reach the established set point. Embryo culture medium uses bicarbonate as a buffering agent which is in equilibrium with the adsorbed CO_2 as follows:



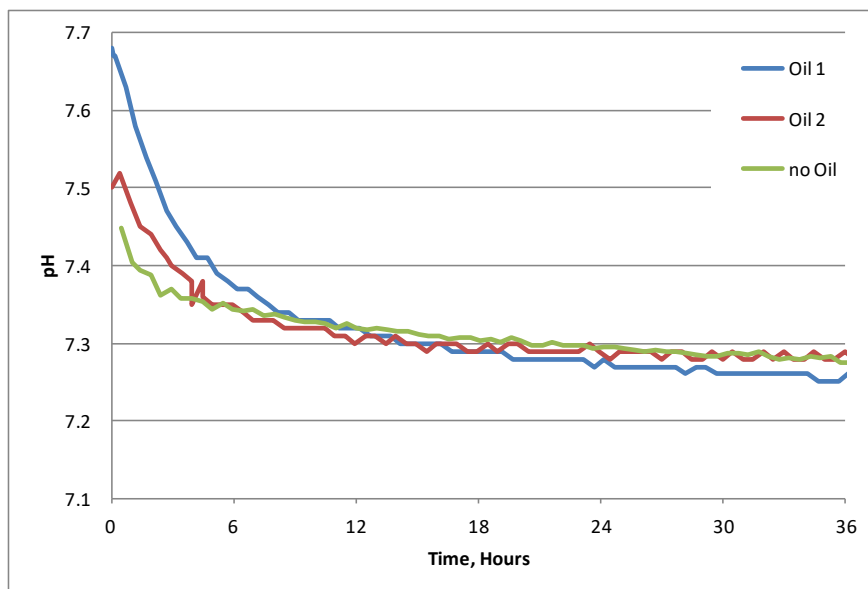
In this equilibrium, raising the CO_2 level will lower the pH and lowering the CO_2 level will increase the pH. Then, while medium is used during embryo culture, environmental changes can cause smaller pH changes. These changes can include temperature and gas fluctuations from opening the incubator door, and even chemical changes due to cellular metabolism. Thus, the ability to monitor pH continually becomes extremely useful because assessment of a single point-in-time sample is not sufficient to adequately monitor pH variations during the culture process. A more complete picture of pH changes over time provides necessary information to identify practices that may be yielding suboptimal media pH and also permit the user to take appropriate corrective actions.



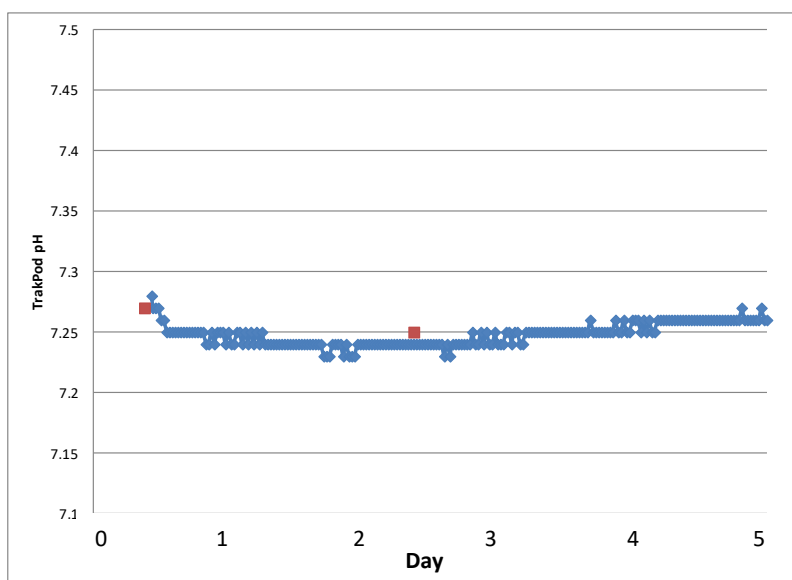
With the SAFE Sens® TrakStation® it is now possible to monitor this dynamic pH profile. We wanted to share some of these pH findings in order to better demonstrate how the incubator equipment plays a role in maintaining pH.

Equilibration

The equilibration time for pH will vary depending on your culture choices. During the equilibration phase, the pH of media changes due to the adsorption of CO₂ through an oil overlay and then into the media. In the figure at the right, the sv² sensors were setup with 100 µL media and 50 µL of oil (this is the standard setup for all other data figures) or no oil. Initial pH and presence and type of oil all impact the amount of time for pH to reach the set point level.



Without oil overlay the media more rapidly absorbs CO₂ initially. Using an oil that is lighter allows CO₂ to penetrate more quickly, but it may also allow evaporation of water from the media to occur (depending on the incubator type being utilized). Oil is essential to slow evaporation of media. The weight of the oil (and volume used) has a trade-off between speed of reaching pH set point vs maintaining pH over the 5-day culturing.



Set Point

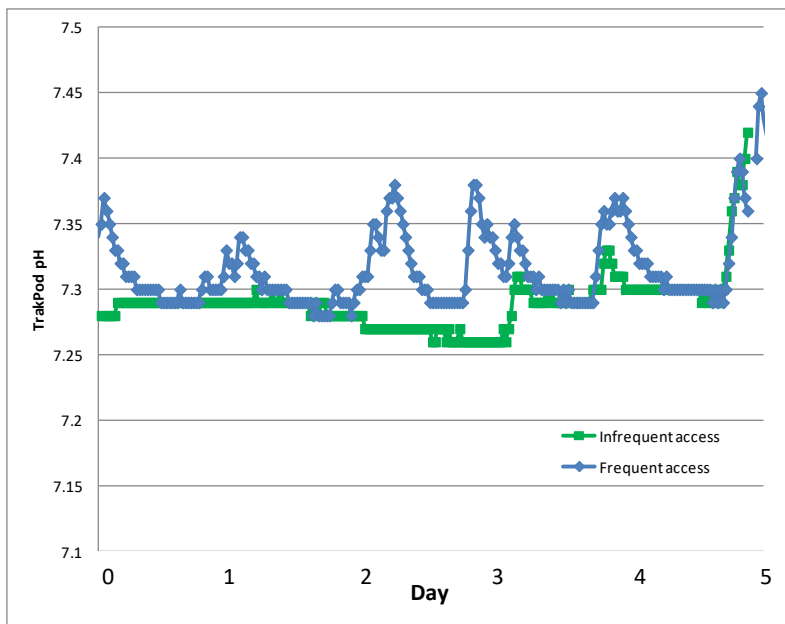
Once media has adsorbed the available CO₂ in the incubator environment, the pH generally stays stable unless there are changes in the environment. One example of a fairly stable pH profile is included here. It includes spot check pH measurements in red as a comparison showing good agreement between TrakPod® pH and manual electrode pH measurements.

Stabilization

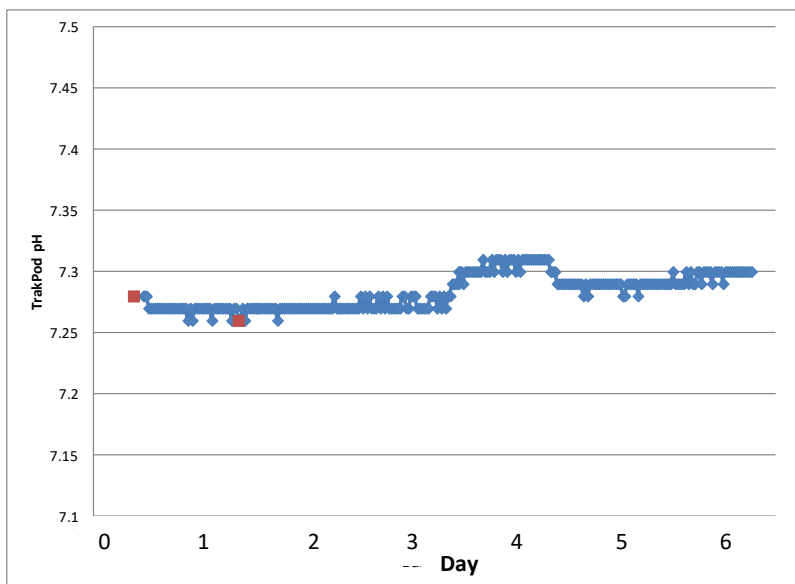
During the stabilization phase pH fluctuations can be caused by incubator door openings, CO₂ set point changes, temperature fluctuations and several other factors. The type of incubator that is used can play an important role in the stabilization fluctuations. Four types of incubators that are commonly used are:

1. Cabinet incubators (typically humidified) which use Thermal Conductivity CO₂ sensors
2. Cabinet incubators (typically humidified) which use Infrared CO₂ sensors
3. Humidified benchtop incubators
4. Dry benchtop incubators

#1. Effect of lab operations on pH profile for Cabinet Incubators with Thermal Conductivity CO₂ sensors. Two (2) incubators are monitored. One with frequent access (blue) and one with little access (green) during a week. The pH increases for blue are in the mornings. Afterwards, the environment takes some time to recover and return to the desired pH level. Because the technology used for Thermal Conductivity CO₂ sensors also depends on temperature and humidity, larger swings in the actual CO₂ can occur.

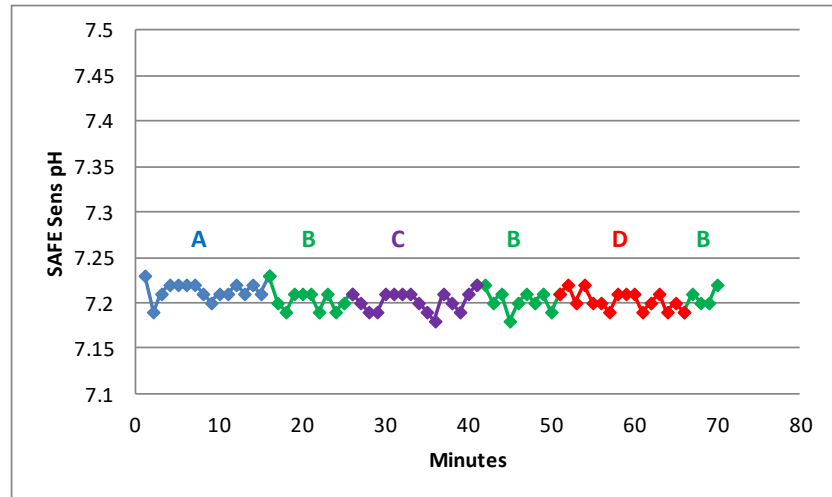


#2. Effect of lab operations on pH profile for Cabinet Incubators with Infrared CO₂ sensors. There is little change in the pH levels during a week of culturing use. This incubator also had infrequent access during the culturing period. The slight pH changes can occur due to shifts in CO₂ or other variables in the incubator environment.

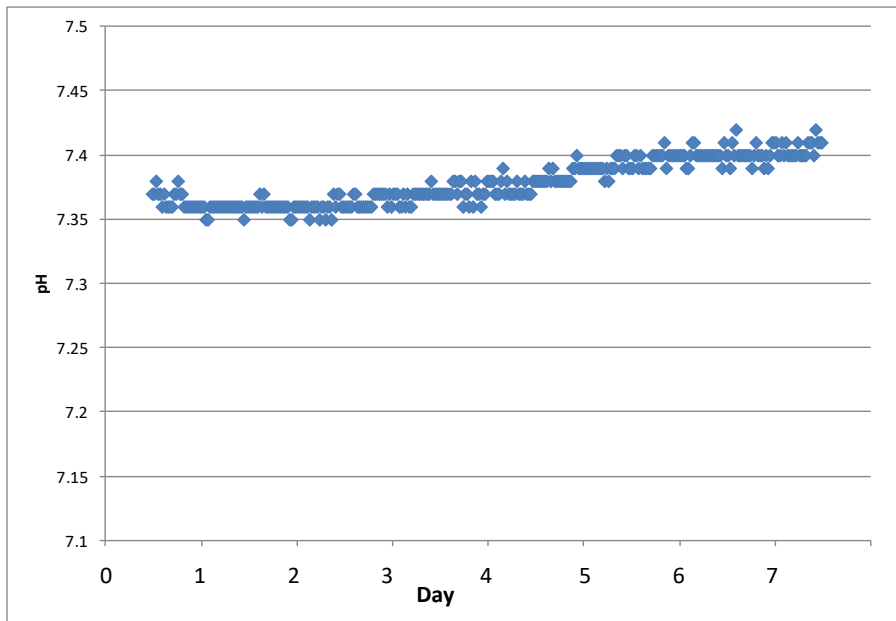




#3 Effect of Bench Top Lid Openings on pH in a humidified benchtop Incubator (Planer BT37). The brief lid openings in benchtop incubators show very small changes in pH, due primarily to the oil overlay of the media slowing any changes in CO₂ content in the media.



15 Minute Lid Open and Close Experiment
 A) Open 30 seconds, close 30 seconds, open 30 seconds, close remainder
 B) Lid remains closed
 C) Open 1 minute, close 1 minute, open 1 minute, close remainder
 D) Open 1 minute, close 30 seconds, open 1 minute, close remainder



#4 Effect of lab operations on pH profile for a dry benchtop incubator (Esco MIRI). Over the course of a week the pH in benchtop incubators stays very steady. The chamber lids were opened briefly (<5 minutes) in the middle of the pH monitoring time. In dry incubators media evaporation can be a concern. The slight pH increase can be due to evaporation of media.

All pH data generated here used 100 µl of media and 50 µl of oil in an sv² sensor. Different culture dish types, media and oil configurations may have different pH behaviors depending on your specific conditions. These data presented in this document are courtesy of:

- Blood Cell Storage, Inc. (BCSI, <http://www.safesens.com/>)
- POMA Fertility (<http://www.pomafertility.com/>)
- Two additional IVF clinics using TrakStation