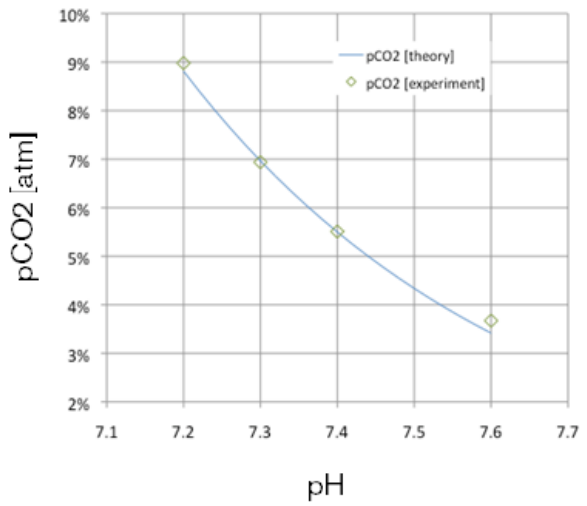
pH validation of culture medium in EmbryoScope+

For optimal embryo development, it is necessary to maintain a proper pH in the growth medium. The optimal pH may differ for different media, but a pH between 7.2 and 7.4 is frequently recommended. For long term incubation of human cleavage stage embryos most IVF laboratories use a culture medium that is based on a bicarbonate buffer system and requires a controlled atmosphere with elevated carbon dioxide (5 – 8%).

The control of the proper pH is mandatory in quality assurance. There are two possibilities to achieve this goal: 1) measuring pH and 2) measuring CO_2. Measuring pH is the only way to detect slight changes between different lots of the same culture media at exactly the same CO_2 concentration.
Here we present a combined method to measure pH and CO_2 in the EmbryoScope+ time-lapse system. Once the correlation between pH and CO_2 of a given media lot has been established, further validations can be done by measuring only CO_2 until a new lot of media is used, requiring a new pH/CO_2 correlation measurement.

Carbon dioxide and pH of IVF culture medium

The pH of the medium is controlled by the carbon dioxide concentration in the incubation chamber. The figure below shows the theoretical relationship between CO_2 concentration in an incubator (in % atm) and the resulting equilibrium pH as a continuous blue curve. The green points are experimental values from *D. Gardner and M. Lane (2000) Embryo culture systems in Handbook of In Vitro Fertilization, 2'nd Ed, CRC Press p. 232-234.*



Calculation based on a medium with a total alkalinity of 27.4 mmol/kg at 37°C, CO2SYS, van Heuven et al 2009: http://cdiac.ornl.gov/ftp/co2sys/CO2SYS_calc_MATLAB/

Carbon dioxide in the incubator is in equilibrium with dissolved carbon dioxide, carbonic acid, bicarbonate and carbonate:

$$CO_2(g) \rightleftharpoons CO_2(aq) \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \rightleftharpoons 2H^+ + CO_3^{2-}$$

The pH of the media at physiological pH can be approximated by the Henderson-Hasselbach equation:

$$pH = pK_a + \log_{10}([HCO_3^-]/[CO_2])$$

The relationship between carbon dioxide concentration within an incubator and the resulting pH in a given medium shows a remarkable resilience to pH change even after substantial change in CO_2 concentration. Large changes in CO_2 thus only cause minor changes in pH. We recommend a stable CO_2 measuring device that gives accurate results in the desired range of 5-8%.

Validation of medium pH for a specific carbon dioxide concentration

It is important to validate the pH of the media being used on a regular basis, to make sure the pH follows recommendations for the specific media. This validation can be performed by measuring the pH of a media sample in a culture dish placed inside the incubation chamber for at least 24 hrs. For this purpose we recommend to fill the EmbryoSlide+ dish with 250µL in each of the two reservoirs under a 1.6mL mineral oil overlay. This will facilitate a sample size of approximately 2x 150µLfor analysis. It is very important to add culture oil in order to avoid evaporation as the environment inside the EmbryoScope+ time-lapse incubator is NOT humidified. The method is described on the next page and requires a blood gas analyzer.

Protocol for meassuring pH/CO2 of culture medium in the EmbryoScope+ incubater

1. **Fill the EmbryoSlide+ dish with 250µL of culture media in each reservoir.**
2. **Overlay with 1.6mL of culture oil.**
3. **Place the dish in the EmbryoScope+ incubator and leave it for at least 24 hours to equilibrate.**
4. **After 24 hours and before opening of any of the incubators: Measure the CO_2 concentration in the incubation chamber of the EmbryoScope+ incubator using a calibrated CO_2 measuring device as described in the User Manual.**
8. **Remove the EmbryoSlide+ dish from the incubation chamber and use the syringe to aspirate medium *without* oil from the dish.**
9. **Transfer sample to a calibrated pH analyzer**
10. **Until a new lot of culture medium is used, regular CO_2 measurements are sufficient as the correlating pH value is known.**

Note

When measuring and interpreting media pH it is important to remember that:

1. The pH-CO2 relationship is altitude dependent. That means that at altitudes higher than sea level, CO2 % must be higher to obtain the same pH.
2. Molecular diffusion through the oil and media layer to reach a stable equilibrium takes several hours. It is thus essential that the media sample is *equilibrated for at least 24 hrs* before a stable reliable media pH can be measured (Einstein-Smoluchowski equation: t=s2/2D).
3. When handling small media samples care must be taken to avoid temperature changes (see figure to the right) and CO2 diffusion into plastware. Pre-equilibration of plastware under CO2 atmosphere may be necessary.The figure (right) shows the moderate effect of reducing media temperature to room temperature while maintaining 5% CO_2. Cooling the media to RT will decrease pH by 0.07 which must be corrected for.