pH and CO_2 validation of culture medium in the EmbryoScope™ time-lapse system

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 cleavage state 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 and 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 to the right 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.*

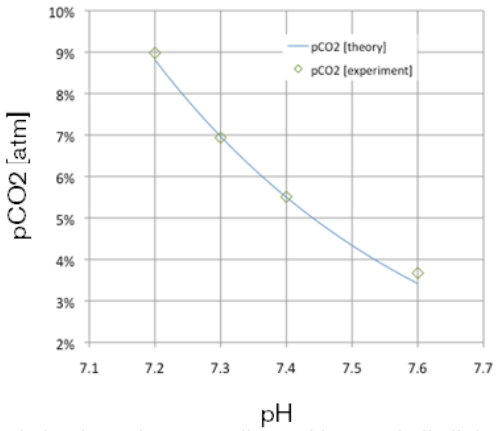
Carbon dioxide in the incubator is in equilibrium with dissolved carbon dioxide, carbonic acid, bicarbonate and carbonate:

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

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. Even though CO_2 concentration measurements in the incubation chamber may vary and be less precise (+/- 0.2%) than pH measurements (+/- 0.05), they are more suited to provide instant feedback and ensure a stable pH during incubation. We recommend a stable CO_2 measuring device that gives accurate results in the desired range of 5-8%.



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/

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 Petri dish placed inside the incubation chamber for at least 24 hrs. We have used 35 mm Petri dishes filled with 3 ml media and with 2.4 ml mineral oil overlay to prevent evaporation. It is very important to add *mineral oil* in order to avoid evaporation as the environment inside the EmbryoScope time-lapse incubator is NOT humidified.

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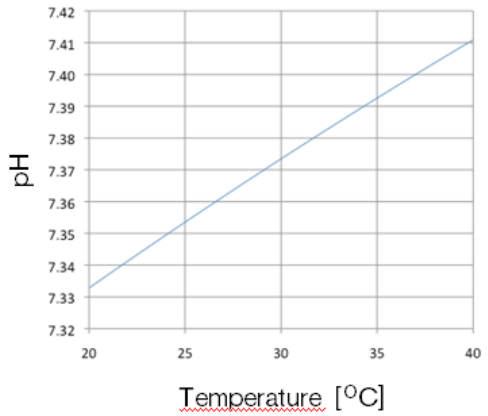
The Petri dish is placed without the lid inside the incubation chamber near the front of the load door on the right side, next to but not covered by the protective cover plate. The Petri dish must be no taller than 12 mm to fit beneath the "tail-fin" of the EmbryoSlide® culture dish. To add a Petri dish: Choose a patient, press "Pause Slide", then open the door, place the Petri dish inside, close the door and press "Cancel".

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

1. Gaseous equilibration is *a very slow process*. 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).

2. The equilibrium constants of the bicarbonate system are temperature dependent. Removing a closed container with media from the incubator and reducing the temperature of the container will reduce the internal pH (whereas any degassing of CO_2 in an open container will increase the pH). The figure below 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. Minor changes in temperature (e.g. of a Petri dish placed on the bottom of the embryo chamber; T=33 °C) can safely be ignored.



Detailed description of pH and CO_2 measurement

The method described below can be applied to pH measurements with standard pH meter with an electrode or with a blood gas analyzer.

1. For both methods: Place an empty tube (lid open) and a 5 ml syringe in a standard incubator for 24 hours to warm up (early in the morning).

2. Place the Petri dish with culture medium (and oil overlay) to be tested in the EmbryoScope time-lapse incubator for 24 hours.

3. After 24 hours and *before opening of any of the incubators*: Measure the CO_2 concentration in the incubation chamber of the EmbryoScope time-lapse incubator using a calibrated CO_2 measuring device as described in the User Manual.

4. Calibrate the pH measuring device using a 2-point calibration (normal pH meter or blood gas analyzer).

5. Remove the 5 ml syringe from the standard incubator and connect a needle to the syringe.

6. Remove the Petri dish from the incubation chamber and use the syringe to aspirate medium *without* oil from the dish.
   For standard pH-meter: remove the tube from the standard incubator, remove the needle from the syringe, slowly fill medium in the tube, and immediately measure pH, while preferably keeping the tube in a tube warmer during measurement.
   For blood gas analyzer: remove the needle and measure directly in the syringe (in case the blood gas analyzer is in another location: cap the syringe and guarantee temperature consistency until measurement is performed).
   Note: It is not recommended to incubate media in a syringe for measurement as this results in altered pH values.

7. Document measurement values for CO_2 and pH; these values do correlate for the specific lot number used.

8. Until a new lot of culture medium is used, regular CO_2 measurements are sufficient as the correlating pH value is known.

Conclusion

Measuring pH is important to guarantee the proper conditions for embryos in any incubator. Although the reliability of pH measurement is controversially discussed, the method that we describe here is based on clinical experience. The correlation between pH measurements and CO_2 for a given lot of culture medium will make this approach very economic, as CO_2 measurements can be performed more readily compared to pH measurements with culture medium. Although equipment for measuring CO_2 is expensive, the savings in culture medium will compensate for the price of this equipment within 1 year based on the assumption of two pH measurements per week.