Effect of weather on validation of CO_2

The purpose of a CO_2 incubator is to ensure a stable environment with constant temperature and pH for bicarbonate buffered culture systems. As will be explained in this technote, maintaining a constant pH requires a fixed partial pressure of CO_2 inside the incubation volume.
The CO_2 sensor of the EmbryoScope time-lapse incubator is designed to measure the partial pressure of CO_2 and regulate the CO_2 intake according to this to maintain the pH at a constant level. When keeping the pH of the medium constant, the CO_2 concentration percentage will change slightly (up to +/-0.2%) with changes in the atmospheric pressure. When validating your incubator for CO_2 using an external device which is pressure compensated you may thus occasionally encounter discrepancies between your measuring device and the reading on the instrument as a result of high or low pressure weather conditions at the site of your clinic.

Understanding the relationship between partial pressure and concentration percentage

All incubators are in equilibrium with the ambient atmospheric pressure. The number of molecules within the incubation volume will depend on the actual atmospheric pressure and will fluctuate accordingly with certain weather conditions. This basically means that at high pressure conditions there will be more molecules inside the incubator, whereas at low pressure conditions there will be less molecules as when compared to normal pressure conditions.

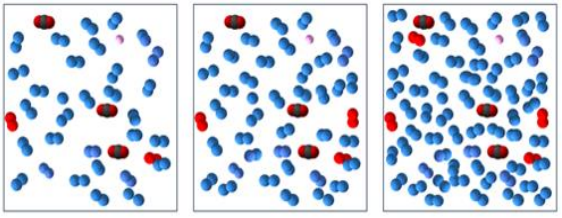

Figure 1. Fixed CO_2 partial pressure at low, normal and high pressure conditions

For IVF incubators, the CO_2 concentration percentage [%] is often used when referring to incubation conditions, which describes the percentual fraction between the CO_2 partial pressure and the ambient pressure $(p_{CO2}/p_{atm})*100\%$. In order to determine the requirements to maintain a stable pH we need to consider the following reactions for the bicarbonate buffer system where CO_2 is first dissolved (1) and then reacts with water to form bicarbonate (2) which can dissociate to hydrogencarbonate and a hydrogen ion (3):

$$(1)\ CO_2(g) \rightleftarrows CO_2(aq)\ ,\ (2)\ CO_2(aq) + H_2O(l) \rightleftarrows H_2CO_3(aq)\ ,\ (3)\ H_2CO_3(aq) \rightleftarrows HCO_3\text{-}(aq) + \mathbf{H^+(aq)}$$

These reactions show that pH in the culture medium is indirectly controlled by the amount of CO_2 in the gas phase. Therefore if you do not want pH to fluctuate with the ambient pressure changes, you need to have the same amount of CO_2 molecules inside the incubator at all times, corresponding to a constant partial pressure of CO_2.

Is your CO_2 setting at the correct value?

Factors such as temperature and pressure affect readings of the CO_2 concentration percentage. This is also the reason that the CO_2 set point of the incubator is dependent upon the altitude of your clinic. The higher the altitude (corresponding to lower ambient pressure) the higher the CO_2 percentage concentration needed to maintain the constant CO_2 partial pressure equivalence at sea level. If a culture media specifies a CO_2 concentration set point of 6.0% at sea level to achieve the correct pH, a clinic situated at 1000m above sea level would need a set point of around 6.8% to maintain the correct CO_2 partial pressure due to the lower atmospheric pressure.

The weather affects the incubator gas composition

CO_2 levels are often measured using a Non-Dispersive Infrared sensor (NDIR). This sensor type is based on the ability of CO_2 molecules to absorb light of certain wavelengths which is directly proportional to the partial pressure. Both EmbryoScope and EmbryoScope+ time-lapse incubators use a NDIR sensor to regulate the CO_2 level based on the measured partial pressure of CO_2. When keeping the partial pressure at a constant level during varying atmospheric pressure (low pressure and high pressure conditions), the percentage of CO_2 in the gas mixture will vary as illustrated in Figure 1. This is depicted as a fixed number of CO_2 molecules being maintained (3 in the image) regardless of pressure. Note that in each case this will result in a different CO_2 *concentration* because the other molecules in the same space become more or less dense according to atmospheric pressure while number of CO_2 molecules remains the same.

TECHNOTE

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Why does my incubator read a different CO_2 value than the validation device?

The EmbryoScope is validated to provide a given absolute concentration percentage at normal pressure at sea level. But what does this mean for your analyzer readings? Let us consider a clinic at sea level as an example. Here a CO_2 concentration set point of 6.0% corresponds to 60.8 mbar CO_2 at 1013 mbar. In this case the display of the incubator will read 6.0% when the CO_2 partial pressure is at the correct value (60.8 mbar). However, if the atmospheric pressure is low, e.g. 980 mbar the actual concentration percentage will be slightly higher (60.8/980) 6.2%. Conversely for a high atmospheric pressure, e.g. 1030 mbar, the actual concentration percentage will be slightly lower (60.8/1030) 5.9%.

This behavior is intentional and both of the above examples with analyzer readings of 6.2% at 980 mbar and 5.9% at 1030 mbar, will end with the result that the pH is maintained constant as intended. It should thus not be a cause for concern, if the incubator display and a pressure compensated validation device report slightly different values when the ambient pressure is away from normal. The table below shows the correlation between the atmospheric pressure and the compensated value you would read on your external analyzer, when your EmbryoScope is properly calibrated to 6.0% at 1013 mbar corresponding to the normal pressure condition at sea level.

hPA/mbar	Compensated device reading*
1070	5.66
1050	5.78
1040	5.84
1030	5.89
1020	5.96
1013	6.00
1000	6.08
990	6.14
980	6.20
960	6.31

** Based on calibration at 1013 mbar at sea level*

When is the pressure change enough to influence the CO_2 readings on my validation device?

The normal range of barometric pressure is from 980 - 1050 mbar. At sea level normal pressure is 1013 mbar. Within this range, the measured CO_2 difference could vary by ± 0.2% based on the weather. If an instrument is calibrated during an extreme pressure system and then a validation check is performed several days later in an opposite pressure system, the values may to appear to be out of specification. If validation with an external analyzer show that the CO_2 sensor requires calibration, we recommend performing this step during normal pressure conditions when using pressure compensated gas analyzers.

Will this affect my culture conditions?

The short answer is – No. To maintain a constant pH simply requires a constant CO_2 partial pressure. The EmbryoScope incubator maintains a constant partial pressure of CO_2 and although the CO_2 concentration percentage of the incubator will naturally depend on the ambient pressure, pH is maintained. In other words this is a prerequisite to keep the pH of the culture environment absolutely stable. It should, however, be noted that small changes in the CO_2 partial pressure (e.g. 0.2%) will only give minute variations of pH (e.g. 0.015). This is illustrated in the graph below, where the pH has been measured for CO_2 concentration percentages in the incubation volume between 4 to 8%. Here it can be seen that CO_2 values of 5-7% are well within the acceptable pH region of 7.2 to 7.4.

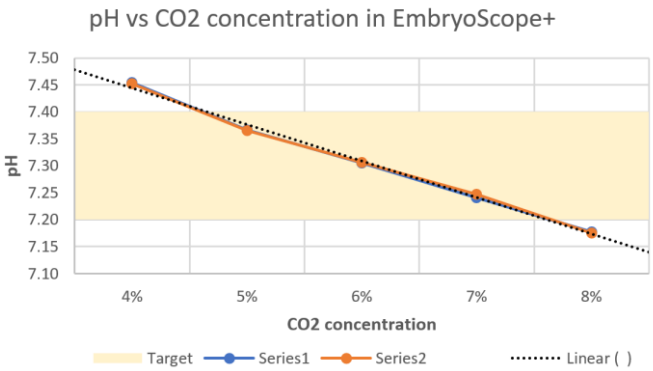

Figure 2. pH measurements of G-TL at various CO_2 partial pressures shown with their corresponding concentrations in the graph.

What does this mean for my Validation procedure?

If you encounter a slight discrepancy between your gas validation device and the EmbryoScope CO_2 reading, or a seeming drift in your CO_2 readings, you should check to see if you have been experiencing an especially high or low pressure system in your area. You should also note pressure systems when calibrating the instrument. If the barometric pressure has been close to normal, then we recommend you perform a standard calibration and adjustment procedure. A pH check is also recommended if the CO_2 percentage is higher or lower than expected based on the weather system.

More about the relationship between CO_2, Pressure and pH is available on our website:
https://www.vitrolife.com/support/support-material

Blog post: Considerations for embryo culture at high altitude
Technote: pH and CO_2 validation of culture medium in the EmbryoScope time-lapse system
Technote: Technote pH validation of culture medium in the EmbryoScope+
Technote: Using the pH validation dish in EmbryoScope+