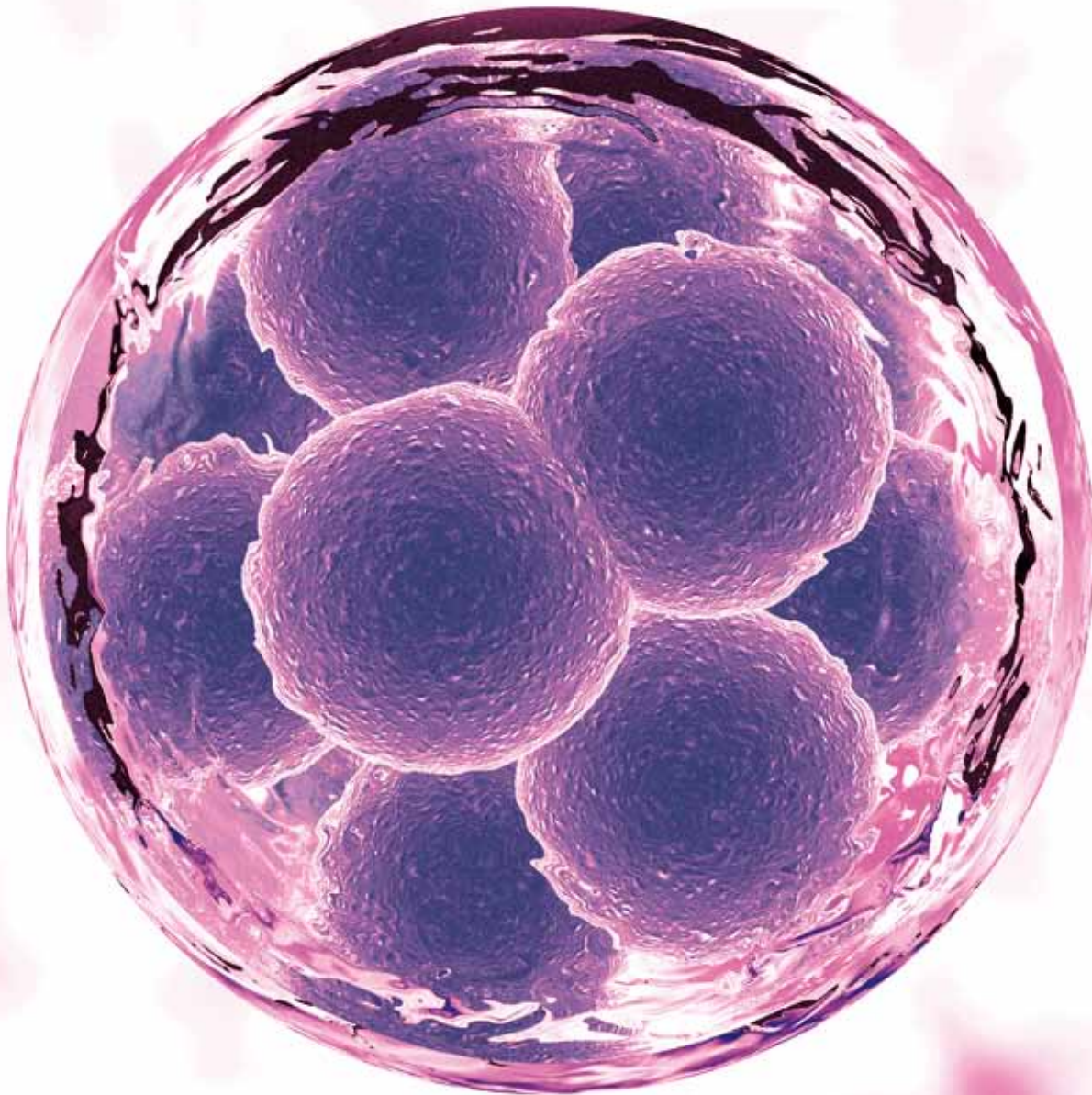


Culture Tips

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pH and embryo culture

pH and oocytes/embryos: (pHⁱ vs pH^e)

Intracellular pH (pHⁱ) regulates protein conformation, glycolysis and a number of other critical metabolic and transport processes in the human oocyte/embryo. Maintaining pHⁱ is imperative to esp. the early embryo.

Extracellular pH (pH^e) in the culture medium is known to be a critical factor in establishing pHⁱ.

What is the natural pHⁱ of human oocytes and embryos?

What should pH^e be in embryo culture media?

What pH^e to culture at (stage specific pH)

A precise optimum pH^e for culture of human embryos has not yet been identified.

The physiological textbook range of 7.2-7.4 is applicable, however there are numerous observations that **differentiated pH** *in vitro* according to developmental stage (oocyte, 2PN, cleavage embryo, blastocyst) may improve development.

Oocytes do not seem to pH regulate to a great extent, and pHⁱ follows pH^e. **Embryos** on the other hand seem to maintain a somewhat acidic pHⁱ (7.1-7.2), even at higher pH^e.

pH during the culture phase should therefore be lowered to target the pHⁱ. pH^e is set slightly above pHⁱ since embryo metabolism also acidifies the cytoplasm.

The most popular pH regime thus describes a high-low-high standard, which seems to be the most beneficial pH standard regime in IVF.

- **Fertilization** Higher pH (7.3-7.4)
- **Cleavage stage** Lower pH (7.2-7.3)
- **Blastocyst** Higher pH (7.3-7.4)

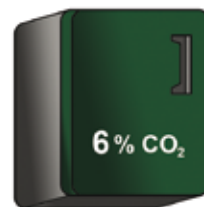
How is stage-specific pH best achieved in culture media?

Choose your culture medium wisely.

The medium buffer determines the equilibrium pH and thus the required CO₂ inflow in the incubator.

Optimal pH control involves CO₂ flow optimized for each medium / embryo stage.

To avoid using multiple incubators for e.g. high-low-high pH regime, choose media with a buffer formulation that complies with the desired pH at a fixed CO₂ level (refer to equilibration charts of each medium, see example p.5).



Fertilization pH 7.3-7.4



Medium 1 pH 7.2-7.3



Medium 2 pH 7.3-7.4

Media buffering characteristics.

By using media with varying bicarbonate buffer concentration, it's possible to obtain varying pH levels using the same incubator setting throughout embryo development

How to measure pH

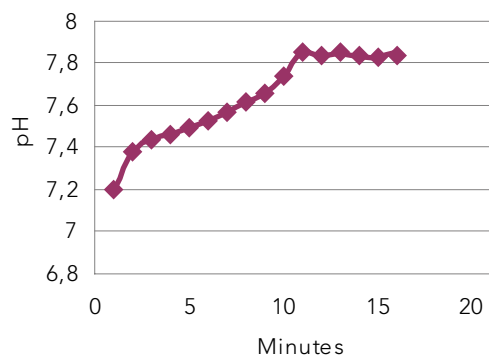
Accurate, reproducible measurements require awareness of several issues, e.g. temperature and protein content of medium.

1. Always calibrate the meter before each group of measurements. Be sure that calibration takes place at the same temp as measurement (e.g. 37 °C) see example on right.
2. Use fresh calibration buffers with one buffer pH close to that of the measured medium. (e.g. pH 7+10)
3. Store the electrode in a designated storage solution.
4. Be aware of probe deterioration. Electrodes deteriorate gradually, and frequently used probes must be renewed regularly, i.e. every ~6 months.
5. When removing CO₂-equilibrated media from the incubator for testing, you must work quickly. Testing must be completed within ~30 sec or the ambient atmosphere will affect pH.

Is your electrode telling the truth?

pH is highly temperature dependent. Below (left) is an example of pH error in a sample medium if the electrode is calibrated at room temp. before measuring pH in the medium at 37°C. Right graph shows pH after placing a cold medium (5°C) in a 37°C incubator.

Calibr. Temp:	22.8°C	37.0°C
Meas. Temp:	37.0°C	37.0°C
CO ₂ :	5.0%	5.0%
pH results:	7.29	7.38



Choice of pH meter:

	Pros	Cons
Standard glass electrodes	<ul style="list-style-type: none"> • Relatively inexpensive. 	<ul style="list-style-type: none"> • Very sensitive to protein clotting. High-protein media such as IVF culture media will quickly coat the electrode and cause inaccurate pH readings. Electrode must be cleansed routinely by ultrasound or protease solutions. • Cannot be fitted inside incubator for real-time measurement.
Microelectrodes	<ul style="list-style-type: none"> • Can be fitted inside incubator for real-time measurement. 	<ul style="list-style-type: none"> • Higher price, same issue as above re: protein in media.
Specialized protein probes (designed to avoid protein buildup)	<ul style="list-style-type: none"> • A more reliable reading. 	<ul style="list-style-type: none"> • Higher price. • Do not completely avoid buildup, i.e. not suitable for ongoing measurements / must still be cleaned routinely. • Cannot be fitted inside incubator for real-time measurement.
Optical pH meter	<ul style="list-style-type: none"> • No direct contact w/medium = free of protein interference. • Will allow reproducible readings, both as single-point measurements and ongoing monitoring. • Can be fitted inside incubator for real-time measurement. 	<ul style="list-style-type: none"> • Higher price.

CO₂ / pH equilibration of MediCult culture media

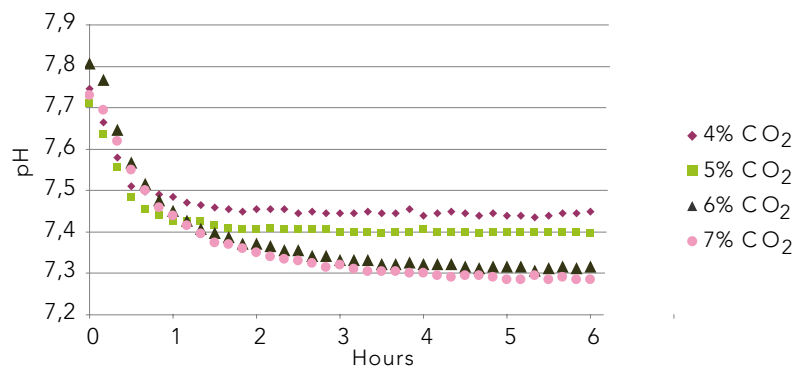
Universal IVF Medium, ISM1™, BlastAssist®

The below graphs depict pH equilibration characteristics of selected NaHCO₃-buffered media from MediCult at 4-7% CO₂. Standard MediCult recommendations are 5-6% CO₂.

Universal IVF Medium

Fertilization medium.

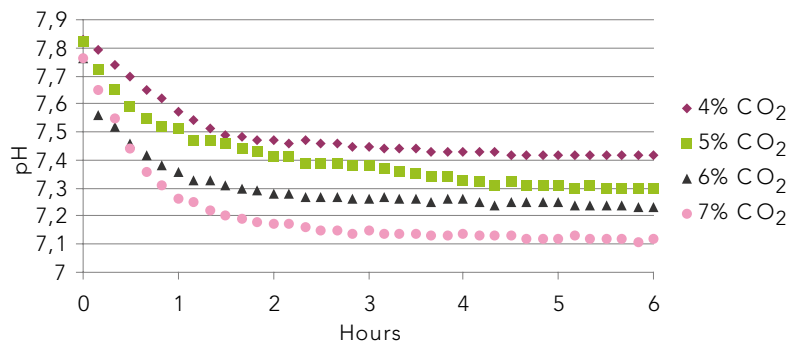
Universal IVF Medium is buffered to achieve a final pH of ~7.3-7.4 at 5-6% CO₂



ISM1™

Early cleavage.

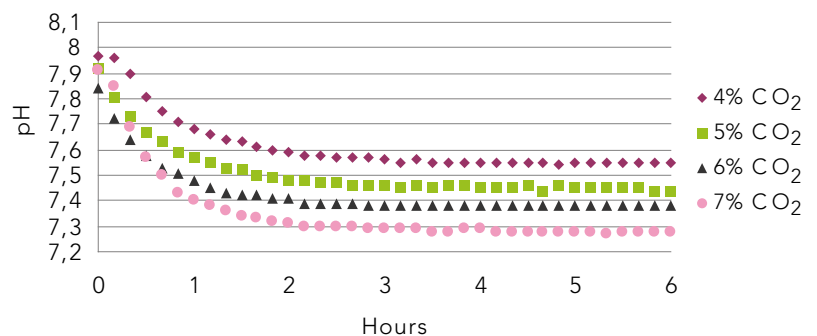
ISM1™ is buffered to achieve a pH of ~7.2-7.3 at 5-6% CO₂



BlastAssist®

Extended culture.

BlastAssist® is buffered to achieve a pH of ~7.35-7.45 at 5-6% CO₂



Temperature control

The oocyte in particular is extremely sensitive to alterations in temperature. Cooling will cause the spindle to depolymerise. When re-warmed, the spindle will repolymerize, but there is a risk of wrongful reattachment of the chromosomes and subsequent aneuploidy of the embryo.

Likewise, the early embryo is more sensitive to temp. changes, which may upset cellular metabolism, membrane stability and transport processes.

Strict temperature monitoring in incubator is advised, and heating stages must be employed whenever working outside the incubator.

Maintaining constant temperature must be a top priority during all stages of culture. Deviations may harm metabolism and mitotic apparatus.

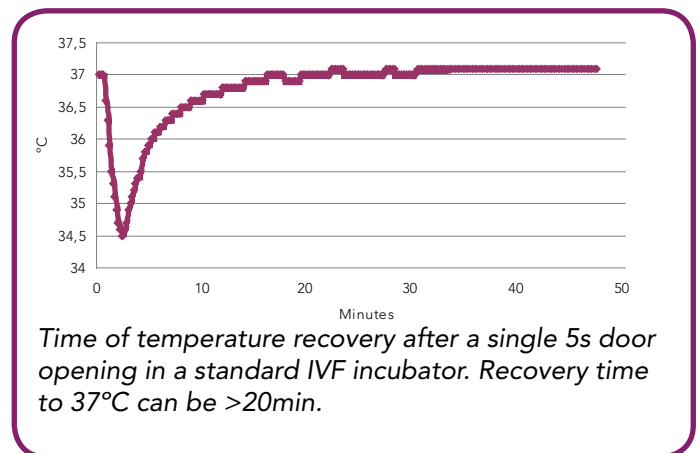
Temperature control during incubation

Door opening reduces incubator temperature. Repeated openings will cause permanent lowering of incubator (and medium) temperature.

Large, multi-patient incubators with recurring door-openings will inevitably affect culture temperature.

Strict temp monitoring and control is advisable. Similar precautions concern CO₂ level and humidity. Loss of incubator humidity will increase evaporation and medium osmolarity esp. when media are without oil cover.

Small (patient-specific) incubators allow much greater control of culture environment.

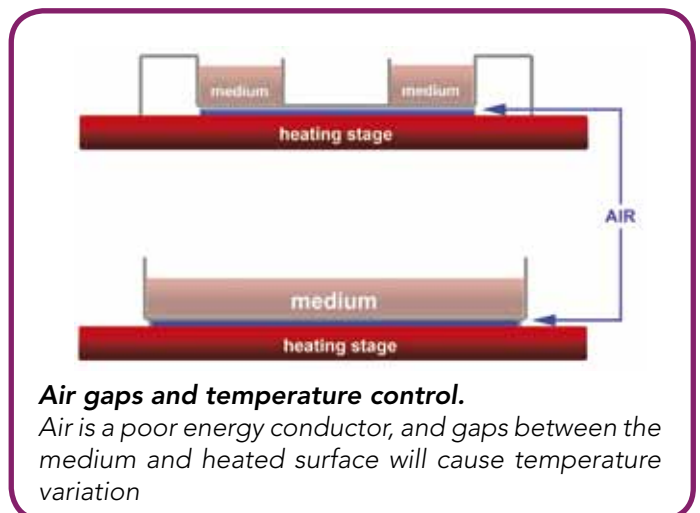


Temperature control outside incubator

Beware that some plastic dishes are designed so that the base of the dish is not in full contact with the heating stage when working under microscope, etc.

Even a small air gap will cause medium cooling which may affect embryos.

Proper warming and quick handling of media outside the incubator is imperative.



Low oxygen culture

In vivo, oocytes and embryos are exposed to a maximum of ~5-8% O₂ in the reproductive system. Atmospheric O₂ may lead to supraphysiological ROS levels, potentially causing oxidative stress (damage to cell organelles, lipids, membranes, DNA, gene expression), and ultimately poor embryo development.

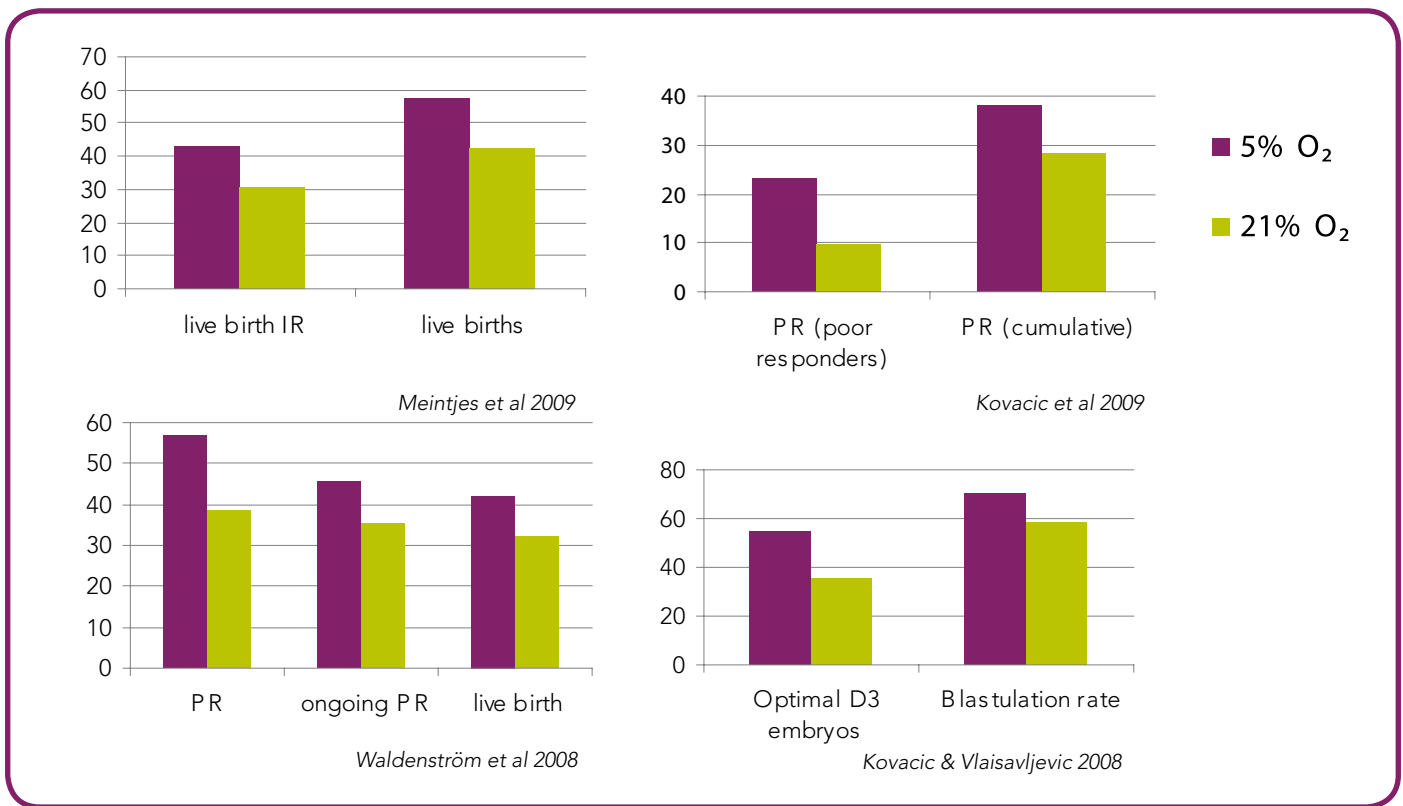
Early testing has been ambiguous, but research from later years has given stronger backing to the concept of lowering oxygen levels during *in vitro* culture.

Greatest effect has been claimed to be in blastocyst

culture, but recent data shows benefit of low pO₂ irrespective of the culture duration (Day 2-Day 6) (Kovacic et al. 2009).

Advanced culture media incorporate a variety of antioxidants/scavengers to counter the hyperoxic effects, but this is a complex issue *in vitro*.

MediCult media are thus formulated for use also at ambient oxygen, but wherever possible, MediCult recommends culture at lowered O₂.



Other culture tips

Air quality

Embryos, esp. cleavage-stage embryos, are sensitive to environmental toxicants, esp. Volatile Organic Compounds (VOC). VOCs originate mostly from vehicles, industrial activity, solvents, etc.

The enclosed incubator environment may have concentrated VOC levels several times higher than the ambient air.

Careful attention should be dedicated to general air supply and purification measures should be taken to effectively remove VOCs – esp. in metropolitan areas, where concentrations can be greatly elevated.



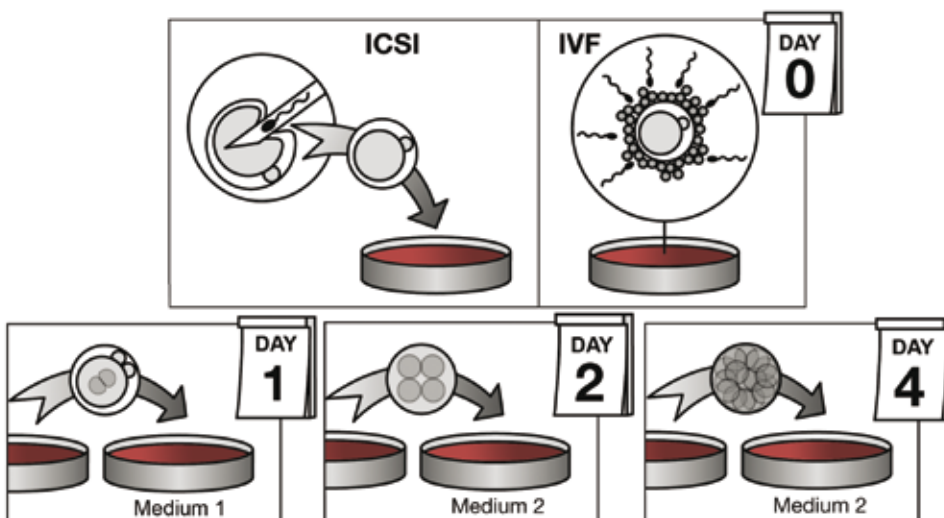
Ensure that air entering incubator (ambient + gas) is clear of VOCs and other contaminants by using the UltraPure 2-stage filter from ORIGIO MidAtlantic Devices.

Day of medium change (extended culture)

Change to stage 2 medium on D2, 3, 4?

Day 2 (4-8 cell) has by some proven to be a better time to transfer to a stage 2 (blastocyst) medium. This is strongly backed by anecdotal evidence and has yet to be confirmed in a controlled trial setting.

Early medium change (Day 2) may avoid interference with the onset of genomic activation (Day 3). The blastocyst culture medium will thus be fully available to the embryo at the time of activation.



Day of medium change.

Following IVF/ICSI fertilization, the 2PN is placed in a stage 1 culture medium. For extended culture, change to the stage 2 medium on Day 2. The medium should be renewed every 48 hrs.

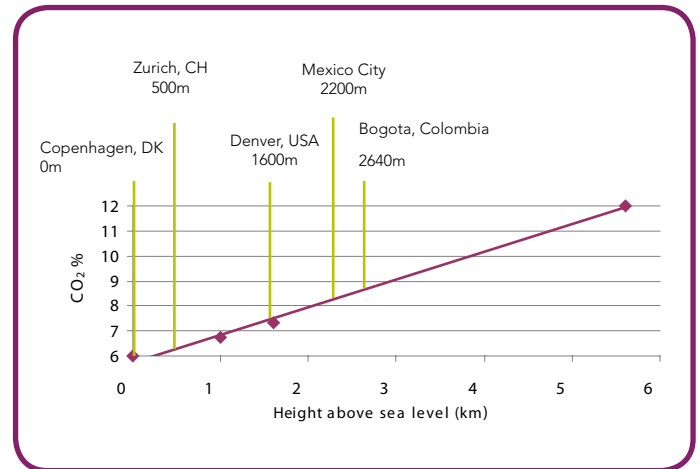
Things to be aware of

Altitude of clinic

Height above sea level influences the required CO₂ level in the incubator.

Cause: Lower ambient air pressure = lower partial pressure of all individual gasses, incl. CO₂.

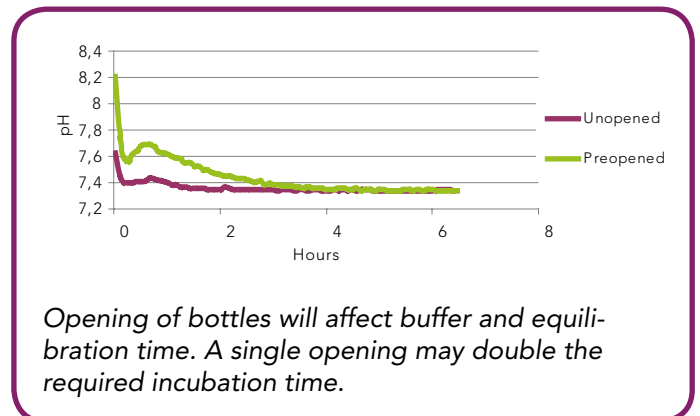
To maintain a fixed pH (e.g. 7.3) in a culture medium, a certain gas pressure is required over the medium. At high altitudes (low gas pressure), a higher CO₂ concentration is required to obtain enough pressure to properly equilibrate with the medium buffer. Example: If 6% CO₂ is required at sea level, >7% is required in Denver, Colorado (see fig on right).



Opening of bottles affects equilibration time

Media from pre-opened bottles may show longer equilibration times vs. unopened bottles.

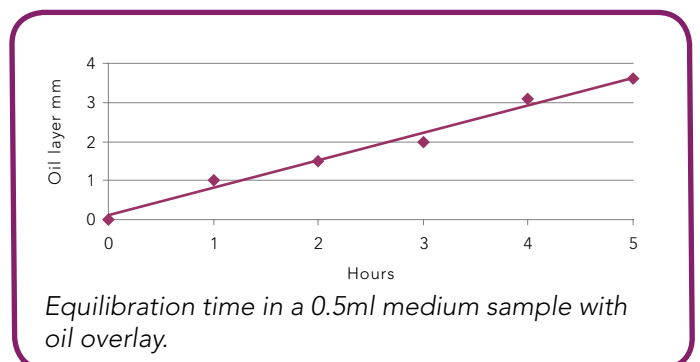
Cause: bicarbonate dissipation from medium in bottle; increased head space in bottle after partial removal of medium.



Thickness of oil overlay affects equilibration

Thickness of paraffin oil added to drops / wells will affect the time to reach pH equilibrium.

Cause: larger exchange barrier for CO₂



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