Media pH measurement in IVF

What is pH and why is it important?

The pH of a liquid is a measure of its acidity. It is measured on scale of 1 to 14 that is proportional to the concentration of hydrogen (H^+) and hydroxide (OH^-) ions that are present in the liquid. Acidic pHs are closer to 1 and basic pHs are closer to 14. The pH is neutral at pH 7.0 when the concentrations of hydrogen and hydroxide ions are balanced. The pH scale is logarithmic; meaning the difference in ionic concentrations between pH 7 and pH 8 is 10-fold.

The physiologic pH of human blood is generally near pH 7.4. All cells growing *in vivo* or in culture media *in vitro* have mechanisms to regulate their internal pH and different cell types and species have different internal pH optimums. In IVF, it is commonly thought that an optimal medium pH is slightly higher than the optimal intracellular pH of the embryos. IVF media manufacturers target their media to maintain pH within a narrow range, commonly between pH 7.2 and 7.4. Typically each medium is crafted to maintain a ±0.10 or ±0.05 pH unit range.

How does the IVF environment impact the pH of my media?

IVF culturing is typically done in conditions that attempt to mimic the conditions inside the female reproductive tract: body core temperature, elevated CO_2 and ideally reduced O_2. These conditions, and especially changes in these conditions, will impact the pH of a medium. Media are typically primarily buffered with sodium bicarbonate, and hence the equilibrium between CO_2 (its "partial pressure" or pCO_2) and the concentration of bicarbonate ions are the prime determinants of the pH of the medium: the more CO_2 present in the atmosphere, the lower the resulting pH of the medium. A decrease in temperature will increase the amount of CO_2 that can dissolve in the medium and also lower the pH.

Because of the impact the environment has on medium pH, it is critical to control that environment with incubators that achieve the intended temperature and gas concentration control, and they must be monitored routinely for proper operation. The target CO_2 level is often chosen with a specific medium pH target in mind. The percent CO_2 can be based on the recommendation from a media manufacturer but the partial pressure of CO_2 is the important consideration for medium pH. As a result the percent CO_2 may need to be adjusted for the altitude of the laboratory and the weather conditions. Temperature is a critical factor in the IVF environment for ensuring normal development of oocytes and embryos, but it is no less fundamental than controlling and monitoring the pH of the medium.

What are the options for pH measurement?

The pH measurement equipment that can be used for IVF culture media fall into four types: blood gas analyzers, electrode pH meters, dipsticks and, more recently: optical pH fluorometers.

Blood Gas Analyzers

Bayer *Rapidlab 348*
(Photo from Reference 1)

Blood Gas Analyzers such as the Bayer *Rapidlab* are primarily used for measuring patient blood, and typically measure pH, pCO_2, and pO_2. Some models include the ability to measure ionic concentrations, as well as the concentrations of glucose, lactate and other substances. Other models, like the Abbott *iStat*, are handheld units. These instruments are costly from a capital investment point of view, as well as sensor and buffer upkeep. Many used and reconditioned models are available.

For IVF, a sample container with the medium or buffer of interest (but no gametes or embryos) is equilibrated under the desired CO_2 concentration and temperature. The sample is then quickly capped, removed from the incubator and brought to the remote blood gas analyzer for sample measurement. Measurement accuracy is predicated on the assumption that no temperature changes or CO_2 off-gassing has occured from the time of removing the sample until it has been read.

Electrode pH Meters

General laboratory electrode based pH meters such as the VWR *SympHony*™ can be used for measuring a wide range of pHs and sample types. These instruments tend to be relatively inexpensive, and are typically calibrated just prior to use with two or three standard buffers. Over time the pH electrodes will lose accuracy when measuring protein rich solutions (but can be cleaned using special solutions and "regenerated").

For IVF, a tube containing about 3 mL of medium is equilibrated under the desired CO_2 concentration and temperature. The sample is then removed from this environment and the electrode is inserted quickly into the open tube to measure the pH. Meanwhile, the sample is cooling and losing CO_2 – both of which impact the measured pH. In some cases, special smaller electrodes can be used and placed inside the incubator. For best results the calibration buffers should also be used inside the incubator environment (although they are only truly "standard" at ambient temperature, around 20 to 22°C). This type of pH measuring device is therefore generally only suitable for cabinet style incubators.

VWR *SympHony*
(Photo from Reference 2)

Research Instruments *pH Meter*
(Photo from Reference 3)

A specific adaptation of the general purpose pH meter from Research Instruments allows a user to place the sensor probe and surrogate media samples directly into the desired incubator environment. The medium can include an oil overlay. For a period of 24 hours the pH can be monitored in this medium sample. The sensor is designed in such a way that it fits into both cabinet style incubators and benchtop incubators. Afterwards, measurement data can be downloaded and saved on a computer.

pH Dipsticks

pH Dipsticks such as the *ColorpHast*® strips are used to determine rough pH in a wide variety of applications from swimming pool maintenance, to beer brewing and semen analysis. The user matches the color of a wet pH strip against the chart on the box to determine pH. Generally gradations between color changes are of the order of 0.3 pH units.

For IVF, a small sample cup or Petri dish containing medium can be equilibrated under the desired CO_2 concentration and temperature. A measurement is then made by opening the incubator and quickly dipping the strip into the container and reading the pH promptly. The medium sample should not be covered with oil as that would also be absorbed into the strip.

EMD *colorpHast* Strips
(Photo from Reference 4)

Optical pH Fluorometers

Two fluorescence based pH readers have been adapted for use specifically for the IVF industry. These implementations include an optical reader and a disposable combined specimen container / sensor with a fluorescent species.

MTG *pH Online*
(Photo from Reference 5)

The *pH Online* from MTG adds a fluorescent sensor spot to the bottom of a special Nunc 4-well dish. The well of the dish is filled with the medium of interest and an optional oil overlay and is placed on a stand with a fiber optic probe located in the incubator environment. The fluorescent signal is read continually at a set frequency and converted to pH values. The pH data are automatically output to a computer where they are graphed and saved in real time. This device is readily suitable for use with a cabinet style incubator and some benchtop incubators include it as an optional add on that must be factory installed. In the benchtop incubators the 4-well plate occupies a significant portion of the available incubation space.

The *SAFE Sens™ IVM* from BCSI has a cylindrical sensor probe in which one end of the sensor has a cup for the medium sample. The cup is filled with a small volume of medium and oil overlay. The sensor is clipped onto a fiber optic probe mounted inside the incubator. The fluorescent signal is read continually at a set frequency and converted to pH. The pH readings are displayed on the reader and automatically output to a computer where they are graphed and saved in real time. This unit is readily suitable for use with cabinet style incubators and the company claims a benchtop configuration will be launched soon.

SAFE Sens IVM
(Photo from Reference 6)

pH Alternatives Summary table

	General pH Technology		IVF Specific Implementations		
	ColorpHast Strips	Medical Blood Gas Analyzer	Research Instruments *pH Meter*	MTG *pH Online*	SAFE Sens
Capital cost	None	$$$$	$$	$$$	$$
Operating cost	$0.15 - $0.50 single test	$2K-5K/year	~ $300/year	$11/dish 1 dish/cycle	$10/sensor 1 sensor/cycle
Sample size	1 drop	> 95 µL	up to 800 µL	50 - 800 µL	50 - 150 µL
Measurement time	5-10 seconds	1-2 minutes	5-30 seconds	1 second	1 second
Continual reading	No	No	24 hours	6 days	6 days
pH measured in incubator	No	No	Yes	Yes	Yes
pH Range	Varies	6.0 – 8.0	0.0-14.0	5.5-9.0	6.0-8.0
pH Accuracy	< 0.5	0.005	0.05	0.03	0.05
pH Resolution	0.3 to 0.5	0.001	0.01	0.01	0.01
User Adjustment	None	Automatic 2 point calibration	Manual 2 point cal, 1 point warm-up	Sensors are Factory calibrated, 1 point manual	Sensors are labeled with a Certification code
Quality Control	None	3 Level QCs	None	None	1 Optical QC
Maintenance	None	Replace electrodes, buffers, tubing	replace pH probe, calibration buffers	None	Replace Optical QC yearly

References

1. http://www.blockscientificstore.com/Bayer-348-Blood-Gas-Analyzer-p/bayer-348.htm
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3. http://www.krackeler.com/products/1251-pH-Strips/21448-ColorpHast-pH-Strips.htm
4. http://www.research-instruments.com/static/products/laboratory-control/ph-meter
5. http://www.mtg-de.com/products/ivf-icsi/quality-control/ph-online.html
6. http://www.safesens.com/

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