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Guide to Accurately Measuring Media pH Within the IVF Laboratory

I. Materials

1) pH meter
   -capable of measuring to at least 0.01 pH units
   -capable of temperature compensation with or without ATC probe

2) Appropriate pH electrode
   -ATC probe if electrode or pH meter cannot compensate for temperature

3) Electrode storage and filling solutions
   - commonly 3M KCl

4) Electrode calibration standards
   -recommend use of standards pH 7 and 10

5) 37°C warming block (optional)

II. Methods

A. Calibration

1) Immediately prior to testing a media sample, calibrate the pH meter according to the manufacturer's protocol. It is important to ensure temperature is compensated for by setting the pH meter to the working temperature (~37ºC) or using an ATC probe warmed to ~37°C. This can be accomplished by placing the ATC probe into a warming block (figure 1A) (*see* **Note 1, 2 ,3**)

2) Aliquot calibration standards into test tubes immediately prior to use and cap. pH standards should bracket your desired pHe value. Recommended standards to use include 7 and 10 to a two point calibration, though the addition of pH standard 4 could also be used for a 3 point calibration if desired (*see* **Note 4**)

3) Warm standards to working temperature immediately before use (~37ºC). This can be accomplished by placing capped tubes in a warming block for ~20minutes (*see* **Note 5**).

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4) Once warmed, place pH electrode into standard pH 7. Allow reading to stabilize and calibrate according to pH meter instruction (~6.98 at 37°). Rinse the electrode with deionized H_20 and gently blot dry. Wiping the probe should be avoided, as it can affect the pH reading. Place the electrode into pH standard 10 and repeat.

5) Once calibrated using a 2 or 3 point calibration, place the electrode back into standard pH 7 and verify reading (~6.98 ± 0.02). If the reading is out of range, repeat calibration (*see* **Note 6**).

B. pH Measurement

1) For culture media used in the incubator, aliquot the media into a loosely capped test tube and place into incubator. Volume of media will vary depending on the size of the test tube used and size of the pH probe/device. Allowing ~18-24h incubation ensures adequate temperature and gas equilibration, though less time may be used for smaller volumes of media if desired (*see* **Note 7**).

   For handling media with HEPES or MOPS, warm to 37°C in a water bath or warming block for 20-30 min in a capped test tube (*see* **Note 8**).

2) Following equilibration of media and calibration of the meter, quickly remove a test sample tube from the incubator and cap. This should be done in the morning before any incubators openings have occurred. Quickly move the tube to the pH meter and place the pH electrode into media. To facilitate rapid measurement, the pH meter should be in proximity to the incubators being used. Allow the reading to stabilize and record the reading. Repeat for each incubator and each media used in that particular incubator (*see* **Notes 9 & 10**).

C. pH Adjustment

1) If pH readings in each incubator are in range (acceptable range set by lab), no further action is required. However, if the pH of media within a particular incubator is out of range, the incubator CO_2 levels should be adjusted according to manufacturer's instructions. Raise CO_2 levels to lower pH and lower CO_2 levels to raise pH to fall within the specified range (*see* **Notes 11, 12 & 13**).

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4. Notes

1. With new pH electrode and meter technology, some set-ups have specialized instructions for use. This protocol details use of a standard bench top pH meter with a normal KCl filled double junction electrode. Variations may be acceptable. These are simply recommendations based on experience.

2. A double junction glass KCl –filled electrode is recommended for use due to protein content of culture media and for use with organic buffers. This helps prevent clogging of the junction and ensures rapid and efficient readings. However, other electrodes will suffice in many cases, though care may be needed in cleaning/restoring electrodes to ensure rapid response and accurate readings. Regardless of the electrode, proper storage conditions and cleaning of electrodes are required for optimal performance. In this case, electrodes should be usually being stored in a 3M KCl solution, not water, and the electrode should remain filled with 3M KCl.

3. Ensure the electrode is filled with the proper solution before use and that sample gate is open if present. It is recommended accuracy of any new electrode be verified against a known standard or electrode before clinical implementation. Some electrodes will yield different pH readings *(28, 29)*, which could be problematic if these readings are used for adjusting incubator CO_2 settings.

4. It is important to use freshly aliquotted calibration standards each time to ensure accurate calibration, as pH of standards can drift following extended exposure to air.

5. Though this step isn't always necessary, doing so ensures more accurate pH readings due to the temperature effect on pH and pH electrodes.

6. When done calibrating, or when electrode is not in use, store the electrode properly according to manufacturer's protocols. This usually entails placing the electrode into a 3M KCl electrode storage solution.

7. Media tested should be exactly what would be used in the laboratory for normal gamete and embryo culture. It is important to use the same basal media, protein source, and concentration to ensure appropriate comparison, as different media may yield a different pHe due to variations in media composition. It is also important to test the pH of handling media (those containing HEPES or MOPS used at room atmosphere) to ensure the pH falls within the correct/expected range.

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8. An important practical consideration regarding pH buffers and their use in IVF handling media entails verifying working pHe. Though many labs measure pHe of their media used within the incubator to help determine the appropriate CO_2 level needed, most do not verify pHe of their handling media utilized for procedures outside the incubator. When using HEPES or MOPS buffered media, one must consider the impact of temperature on pH. Raising the temperature of media containing a buffer like HEPES or MOPS results in a corresponding decrease in pH.

9. It is helpful if there is a tight fit or seal between the electrode and the walls of the test tube. This can be accomplished by fitting a rubber gasket around the electrode to fill the gap when placed into the test tube to help stabilized the reading. Alternatively, a small hole can be made in a test tube cap and the electrode slipped through this hole. The cap/electrode can then be fitted onto the test tube to create a seal (Figure 1B). This setup can be used during electrode storage, and if the same size test tubes are used, the cap/electrode can be easily fitted over the test sample test tubes to create a seal and help stabilize pH readings.

10. Two test two tubes per incubator per media can also be used and the average the two readings recorded if increased accuracy is desired.

11. The first step in monitoring pH of media within the IVF laboratory is to first set an acceptable target range for pH in your lab. This range should be narrow to help ensure optimal quality control. There is likely not optimal a pH, but certainly a narrow range is beneficial in reducing lab variability. A range of 7.27-7.32 at 37ºC is a good starting point, but it is recommended that labs following recommendations given for a particular media by the manufacturer.

12. Readings can be performed on a weekly or daily basis based on lab protocols. Weekly measurement may be more practical when one considers cost of culture media and other variables.

13. Importantly, if testing a new lot of media, perform the pH reading in a side by side fashion with the old verified lot. If the pH of the new lot is out of range and cannot be easily brought within range by a small adjustment of CO_2 levels, repeat measurements to verify before contacting the media company to request a new lot of media. All new lots of culture media should have pH verified before clinical implementation.

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Figure 1: A) Photograph of a set-up used to measure pH of culture media. The pH meter is outfitted with both a double junction glass electrode, as well as an ATC probe to compensate for temperature. A warming block is used to maintain temperature of samples for accurate calibration and readings. B) The electrode has been fitted through the cap of a test tube to ensure a tight seal and to help stabilize pH readings when samples are removed from the incubator.