

pH EQUILIBRATION IN 2 DIFFERENT EMBRYO CULTURE DISHES

1) INTRODUCTION

Within the field of IVF, plastic well dishes such as the Minitüb 5-well-dish and the Nunc 4-well-dish are used for the purpose of embryo culture. Apart from the composition of the media and the temperature, the pH and its stability are a decisive factor for the in-vitro development of embryos and the success of the overall culture. Embryologists know it takes time for the pH value to stabilize in a freshly prepared culture dish. It has been determined that the equilibration period is influenced by many factors including the physical properties of the culture dish itself. Testing has shown that the distance between the lid and the body of the dish can enhance the exchange of gas and therefore affects the rate at which pH stabilizes. For the embryologist a short pH equilibration time can be advantageous to the overall culture.

The aim of the below study is to identify the time in which the pH value of the media stabilizes after dish preparation. Both the Minitüb 5-well dish and the Nunc 4-well dish were tested in this pH analysis.

a) Minitüb: 5-well-embryo culture dish (Ref. 19021/0001)



b) Nunc: 4-well-multi dish



2) PROCEDURE

To continuously measure the pH value in the media over a time span of several hours, the pH Online™ 1, from MTG Medical Technology Vertriebs-GmbH was applied: (<http://mtg-de.com/phonline.html>).

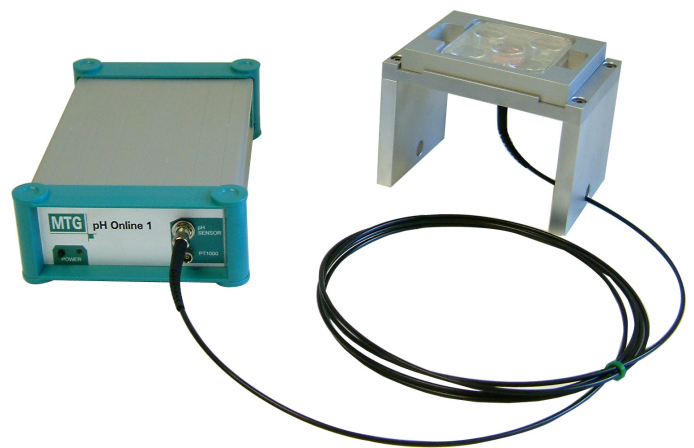


Fig.: pH Online™ 1 with 5-well-dish

The pH measurements in both dish types were carried out in an incubator (RS Biotech Micro Galaxy (14 litre)) at 37°C and 5.3 % CO₂.

One well of each dish was filled with 700 μ l Irvine Scientific HTF Media, supplemented with 10 % (Vol/Vol) SSS. The open dishes were exposed to room air for 20 mins without oil overlay in order to increase the pH of the media. Afterwards, the media was covered with 400 μ l sterile mineral oil (Oil for Embryo Culture, Irvine Scientific). The dishes were then put into the incubator and the pH of the media was measured every 5 min over a time span of 20 hours. To achieve this measurement each dish was equipped with a pH Online Sensor.

A total of three dishes from each dish type were tested using the above procedure.



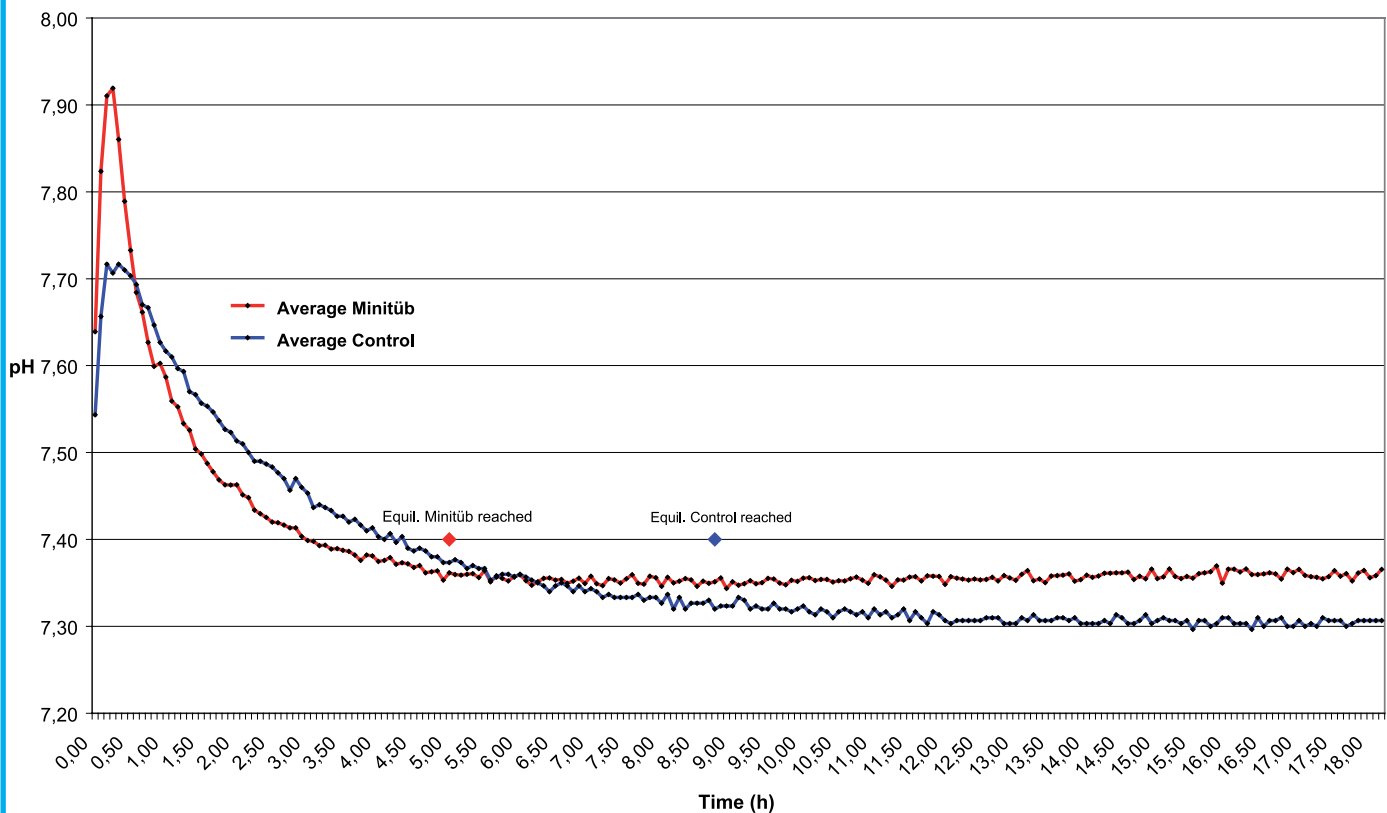
3) RESULTS

A stable pH value was achieved approximately 5 hours after preparation within the Minitüb 5-well dishes. The Nunc 4-well-dishes took an average of 9 hours to achieve equilibration (see diagram).

The shorter equilibration time seen in the Minitüb dishes can be contributed to the larger diameter of the wells: The

contact surface between oil/media and the atmosphere in the incubator is 30% larger in the Minitüb 5-well-dishes than in the Nunc 4-well-dishes. This is also expressed in a stronger rise of the pH during the exposure of the media to the room air.

Time until equilibration: Mean value for 3 runs



4) CONCLUSION

After a considerably shorter pre-incubation time, the Minitüb 5-well-dishes equilibrate to a stable pH value faster than the Nunc 4-well-dishes.

Execution of the test: Dr. Paul Gassner, MTG Medical Technology Vertriebs-GmbH (<http://mtg-de.com/>).