Performance characteristics of the MINC+® Benchtop Incubator

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Introduction

The embryo culture 'incubator is arguably the most important piece of equipment in the laboratory.'[1] Embryo culture incubators should provide a consistent internal environment in order to stabilise culture variables such as pH and temperature. In addition, an ideal incubator will return internal conditions quickly to equilibrium values after a disturbance, such as opening the door to retrieve a culture dish. These factors are essential to consider when determining the specific incubator to use for human embryo culture.

More than 20 years ago, Cook Medical introduced the first benchtop embryo culture incubator, the MINC® (mini-incubator). Its design features were revolutionary at the time and included a small inner chamber, direct heating of the dish, and active humidification via forced gas through a humidification flask. These design features allowed the system to achieve the ideal parameters described above. Since this time, the MINC has become the standard against which other benchtop incubators are measured.

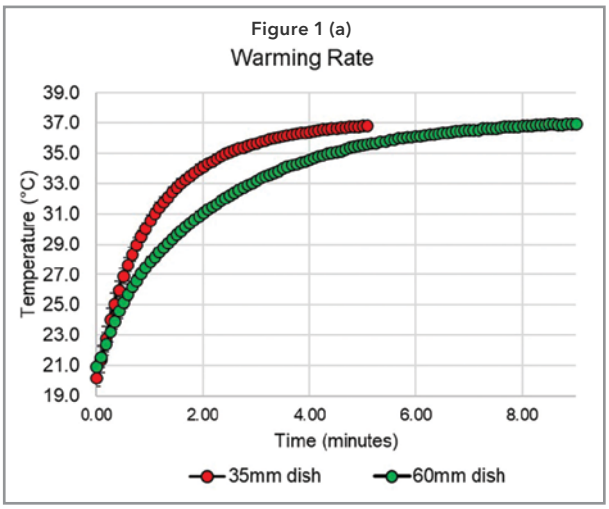
As of 2022, an improved incubator that maintains all of the benefits of the original MINC, and includes many improvements, is being offered to embryo culture laboratories: MINC+. Several important performance characteristics are detailed below, with associated discussion regarding why we feel that the MINC+ is the premier incubator available for IVF.

Temperature dynamics: initial equilibrium and effects of lid opening

Since culture stability is critical for the success of IVF, important questions arise when evaluating incubators: **When dishes for embryo culture are made and placed inside the incubator, how long does it take for the medium to reach equilibrium? How does removing a dish from the incubator into room temperature for routine observations affect the temperature of the culture medium? How fast does it recover once the dish is replaced into the incubator? Experiments were conducted to address these questions, and the results are presented below.**

Part 1: Warming of a dish with medium and oil from ambient temperature to 37 °C when initially placed into the incubator.

This study was designed to assess the time necessary for a dish with medium and oil to attain the setpoint of the MINC+ (37.0 °C) when the components start at ambient temperature (20–21 °C) and are then placed inside the incubator chamber. Figure 1 shows the dynamics of this temperature change in 30 µL medium drops covered with a 3 mm oil overlay in 35 and 60 mm dishes.



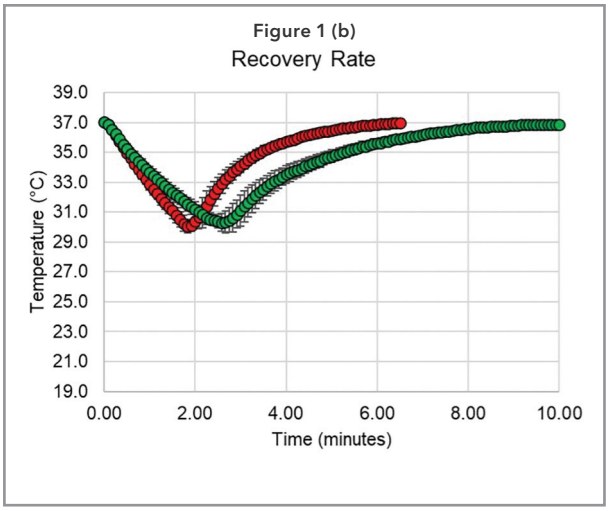


Figure 1 shows the temperature dynamics of a 30 µL drop of medium covered with oil in a 35 or 60 mm dish starting at room temperature (20–21 °C) and moved into the MINC+ when the setpoint of the MINC+ is 37.0 °C (a). The data show that the temperature rapidly rises and approaches equilibrium (~ 37 °C) in an asymptotic fashion. Removal of the dish into a room temperature environment for 2 to 2.5 minutes results in the temperature dropping by about 7 °C (b), and then, after placing the dish back into the incubator chamber, the temperature exceeds 36 °C within 2.5 minutes, on average, (35 mm dish) or 4 minutes (60 mm dish), and reaches 37 °C in approximately 4 minutes (35 mm dish) and 7 minutes (60 mm dish). A 40 Ga Type T thermocouple junction was located in the medium drop throughout these readings. The thermocouple was obtained from Omega Engineering (Norwalk, CT USA) and validated against a Fluke 5610-6-P precision thermistor. Temperature logging was achieved using an Extech Instruments TM500 12-channel Thermocouple datalogger (Nashua NH USA). Temperature data was captured once every 5 seconds. Mean ± 95% confidence intervals are shown.

Part 2: Temperature loss upon lid opening when a dish remains on the warm surface of the incubator.

Figure 2 shows the temperature loss for microdrops in a 35 mm dish prepared as described previously, when the lid of the MINC+ is opened for varying amounts of time, with the dish remaining in the incubator. For the 35 mm dish (2a), when the incubator lid is held open for 5 seconds, the temperature drops by only ~ 0.1 °C. Even a 25 second duration causes minimal loss of temperature, with it dropping by only 0.1 to 0.2 °C. The temperature loss is similar for a drop in a 60 mm dish, with a drop in temperature between 0.0 and 0.2 °C up to 25 seconds.

Figure 2

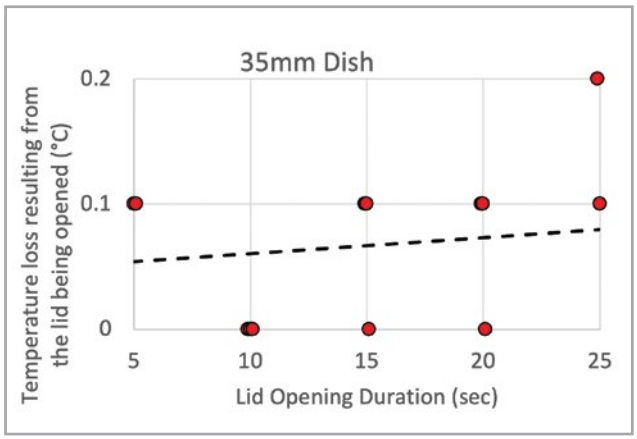


Figure 2. The graph shows the relationship between the reduction in temperature of a culture medium drop in a 35 mm dish when the lid of the MINC+ is held open for varying amounts of time, with the dish remaining inside of the incubator. A slight dependence of the temperature decrease as a function of time was seen with regression analysis (dashed lines). Temperature measurements were obtained as described in the legend for Figure 1 and collected at a rate of one reading per second. Several of the data points had identical values (e.g., all 3 of the points at 10 seconds had a value of 0 °C). To distinguish the points, some of the time values were offset by 0.1 °C to show multiple points at one place on the graph.

Gas dynamics: recovery of CO_2 concentration upon lid closing

How fast does the gas concentration recover inside the incubator after the lid has been opened and closed?

Figure 3 shows the CO_2 concentration measured inside the incubator after opening and closing the lid. Measurements were taken at 6 different positions within the incubator. The average and standard error of the 6 measurements are presented. The concentration approaches equilibrium (6.43%) asymptotically and is greater than 95% of that value by 3 minutes.

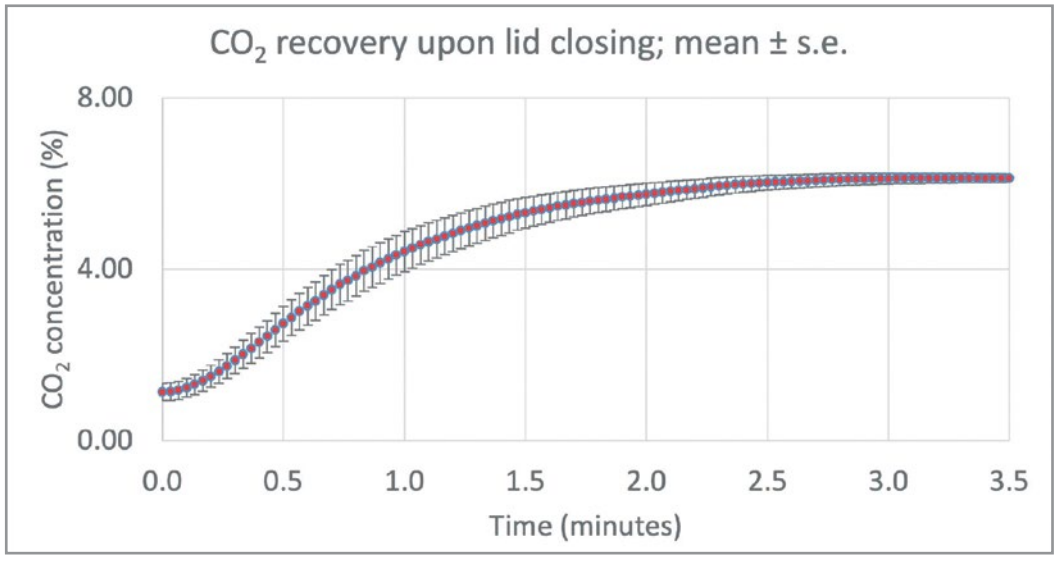


Figure 3. The dynamics of CO_2 concentration inside the chamber of the MINC+ after opening and closing the lid are shown. The data represents the mean ± standard error at 2 second intervals. Carbon dioxide concentrations were measured using a calibrated K30 10% CO_2 sensor and Gaslab software from CO2meter.com at 2 second intervals. The CO_2 concentration of the gas source was measured at 6.43% and is used in our laboratory to maintain the medium pH at a desired level (see reference 3 for a thorough discussion of pH in embryo culture).

Discussion

We have provided here an overview of some of the performance characteristics of the new MINC+ Benchtop Incubator offered by Cook Medical. Cook has a deep history in the area of clinical in vitro fertilisation and appreciates the importance of consistency for quality. The MINC+ was designed to provide the same level of stability and performance as the original benchtop incubator, the MINC, but with added features and benefits.

It is generally considered important to maintain a controlled environment for human embryo culture to achieve optimal results.[1,2] This includes parameters such as temperature culture media pH, and osmolality. Therefore, the choice of which embryo culture incubator is paramount for the establishment of an excellent embryo culture system.

Here we describe the results of studies designed to assess the performance characteristics of the new MINC+ Benchtop Incubator offered by Cook Medical.

In the first study, we assessed the rate of temperature change from ambient to 37 °C, and also from 30–37 °C, the latter being associated with removing the dishes from the incubator for 2–2.5 minutes, as one would do to perform a check on fertilisation and embryo development. The time necessary to return to thermal equilibrium after opening the lid is similar to the time assessed previously with the original MINC incubator.[4,5] These results were not surprising, considering the same heating system is used in the MINC+ incubator as was employed in the original MINC incubator.

The second study was designed to assess the temperature loss of a drop of medium in a dish when the dish remained on the surface of the incubator when the lid was opened for an expected time duration necessary to retrieve a dish from the incubator (as determined during this study and also by others).[5] Considering the very effective method of temperature maintenance in the MINC incubators, only a very small effect of lid openings on drop temperature was detected. In a 35 mm dish, the temperature only decreased by 0.1 to 0.2 °C for lid opening durations between 5 and 25 seconds. In the study investigating the same effect in a 60 mm dish, the temperature loss was the same as the 35mm dish (0.2°C).

Temperature is one important parameter in an embryo culture system. Temperature fluctuations can result in detrimental effects to the meiotic spindle in mature oocytes,[6] developmental kinetics, blastocyst quality, and metabolism.[7] These results demonstrate the value of the superior temperature control system employed in the MINC+ incubator, as in the original MINC. Removing dishes from the chamber of the MINC+ for assessment will have a negligible effect on the temperature of the media in other dishes remaining in the chamber.

The third study presents further evidence that the MINC+ design is robust in the maintenance of the embryo culture environment during routine use. Upon lid opening and subsequent closing, the CO_2 concentration returned to near-equilibrium values in approximately 3 minutes. The kinetics of such a recovery should minimise the pH fluctuations in the culture medium resulting from lid openings.

Maintenance of medium pH is important for proper embryo development.[8] This may be particularly true for the earliest stages of preimplantation development, as unfertilised oocytes from several mammalian species lack some common ion exchange mechanisms to regulate intracellular pH,[9,10] and this is apparently not restored until several hours after fertilisation.[11] Furthermore, the medium pH is a critical parameter for fertilisation, with slightly alkaline pH optimal in many mammalian species.[12,13,14] It should not be forgotten that the bicarbonate ion is also a critical component of some fundamental processes, including sperm capacitation,[12,15,16] and these variables are often confounded in studies attempting to understand the effects of one or the other parameter on fertilisation and embryo culture.[8] Taking into consideration the importance of pH as a variable in IVF and embryo culture and using an incubator that maintains stable levels of CO_2 and returns the CO_2 level quickly after disturbance is critically important.

Previous data investigating the effect of removing a dish containing medium under oil on the medium pH showed that moving from the relatively high concentration of CO_2 in the incubator to the very low CO_2 concentration of the normal atmosphere for 10 minutes has a very small effect (pH change from 7.33 to 7.37).[3] They reported that removing the dish from the incubator for 1, 2, or 5 minutes had no measurable effect on pH. If a dish is to be retrieved from the MINC+, the chamber only needs to be opened for a very short time (on the order of 5 seconds). During that time, the CO_2 concentration surrounding the dish will drop rapidly, but will be re-established relatively quickly (within only a few minutes as shown above). Based upon the data from reference 3, this suggests that opening the lid on the incubator to retrieve a dish will have no measurable effect on the pH of the medium in the dishes that remain in the incubator. This, combined with the fact that the temperature of the medium undergoes a very small change with a 5 second lid opening event (on the order of 0.05 to 0.1 °C), suggests that chamber openings will have a very small effect on the conditions of the embryo culture medium for dishes not being removed from the chamber.

Cook Medical is launching an updated version of its benchtop incubator, the MINC+. The data described above show that the new MINC+ incubator provides confidence in maintaining crucial variables during embryo culture, and hence, confidence in its use in clinical IVF.

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