BENCHTOP INCUBATORS — TIPS & TRICKS

Preamble
Before founding Oozoa I was Scientific Director of Sydney IVF where, in the mid-1990s, I initiated the development of what became the Cook MINC benchtop incubator. All Oozoa-managed labs, and many of our clients’ labs, since that time have exclusively used benchtop incubators for embryo culture. It has been most gratifying to see not only the global expansion in labs’ use of benchtop incubators, but also more recently the burgeoning of models – at ESHRE 2012 we saw benchtop incubators from at least 7 companies.

Wider use has revealed some issues regarding the proper use of such incubators, and this Tips & Tricks highlights some of the more important factors that can affect their proper use. Their compact size brings many advantages, but also some aspects that, although not actual disadvantages, are rather different considerations to using “big box” incubators. While the advice is largely based on our over 15 years of experience with the Cook MINC, we have changed to using the Planer BT37 over the past couple of years.

Location, Location, Location
The location of benchtop incubators within the IVF Lab is extremely important. Proper movement of air around them is essential for heat dissipation to prevent over-heating, but cold air draughts from the HVAC system must be avoided. Why? Because while big box incubators use a lot of power to keep themselves heated, and water jackets on older models were especially effective at minimizing the effect of external temperature fluctuations, benchtops employ lower capacity heating systems that reduce their power consumption, make it easier to run them on battery backup, and reduce the emergency generator load in case of power failure. They generally have lower thermal mass and rapid reaction digital temperature control, enabling tight control of temperature (see the BT37 thermal map on the Planer website).

The lid of the incubation compartment must be actively heated (unless it is a long way from the top of the dish) to avoid condensation forming on the underside of the Petri dish lids. But the humidification compartment lid isn’t heated in the BT37 or MINC, so cold air draughts can cause undue cooling, leading to an increased risk of condensation forming in the gas lines (see below).

So make sure that all benchtop incubators are located away from the direct flow of air from A/C units or HVAC supply ducts. If this is not feasible then protect the incubators using deflectors attached to or suspended below the air vents, or even install a protective perspex canopy over them. This might seem a nuisance, but the proven clinical benefits of benchtops over big box incubators, as well as space saving, make it definitely worthwhile.

Don’t Get Overheated
Incubators can only operate effectively within a certain ambient temperature range. This is because the temperature control uses a servo system that includes heating but no active cooling. The heating phase is governed by the internal heaters whereas the cooling phase of the cycle relies solely on heat dissipation to the ambient background, and is governed by the differential between the chamber temperature and ambient – so if the lab is too hot the unit can’t cool and becomes overheated.

Common causes of temperature control failure include:

- Exposing the units to external sources of heat such as direct sunlight (also remember that the sun comes from different directions during the day, and also changes with the season).
- Placing documents (e.g. a file or clipboard) on top of the incubator: heat loss through the lids is part of the heat dissipation model.
- Leaving an incubation compartment lid open for an unnecessarily long time when accessing dishes.
- A cold lab if the air conditioning is programmed to go off at night.

An advantage of the BT37 in that its internal fan exchanges air with the environment (so keep the air filters clean!), increasing heat removal and allowing the unit to operate in warmer labs than the MINC.

Basically, do not allow the lab temperature to even approach the incubator manufacturer’s stated maximum, and ensure there is sufficient general air movement around the incubators.

Don’t Be A Klutz
Installing the humidification bottle and tubing sets can be fiddly, especially for male embryologists with larger fingers, but it is vital that they are installed carefully so as to avoid possible kinking of the tubing that will prevent gas flow.

- When connecting the 0.22 μm filter to the inlet inside the humidification compartment remember to rotate the filter a half-turn anticlockwise before twisting it onto the Luer-Lok connector. This will prevent the attached tubing becoming twisted as the filter is screwed onto the Luer-Lok connector, blocking the gas flow.

- When installing the two tubes from the humidification bottle to the incubation compartments be very careful not to twist or kink them, and not to compress them when inserting them into the metal grooves as this will block gas flow.

Installing humidification sets might appear tricky, but it is technically very simple and (after a little practice) should be no real issue for embryologists who routinely perform as skilled a technique as ICSI. As with any critical action, when a bottle set has been changed double check that everything is correct before returning the incubator to regular operation (also see “Changing humidification sets”, below).

Avoiding Condensation
After the pre-mixed gas is humidified by bubbling through the water in the humidification bottle it must pass through lengths of tubing into the incubation compartments. But if the gas cools during this passage some of its humidity will condense as water along the inner surfaces of the tubing. As this water accumulates it will form droplets that can, eventually, block the tubing, blocking gas flow. Even on purge, gas flow rates are likely insufficient to clear such a water droplet by blowing it through the tubing into the incubation compartment. There are several aspects to minimizing the risk of condensation and avoiding the problem of gas flow failure:
The humidification bottle temperature is cooler (by about 2°C) than the incubation compartment.

Prevent the humidification compartment from cooling: don’t open the lid unnecessarily and avoid draughts cooling its cover.

The backlit observation window of the BT37 precludes the need to open the compartment to check the bottle.

The BT37 has two additional, patented, features to reduce the risk of condensation forming inside the gas lines between the humidification bottle and the incubation compartments.

**#1** A black metal block with grooves (the “fish block”) helps keep the tubing warm to reduce the risk of humidity in the gas flow condensing out as water droplets. Insert the two supply tubes very carefully, with no twists, kinks or compression, especially where they cross.

**#2** A perspex inner cover prevents the entry of cooler room air into the compartment when its cover is opened to check the bottle or gas lines.

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### Use the Right Gas

- Be sure to use the correct gas mixture. For most human IVF and embryo culture media this is 5.8–6.0% CO₂ / 5.0% O₂ / balance N₂ at sea level (although 5.0% CO₂ should be used with Sage media). At higher altitudes increase the %CO₂ to maintain the correct partial pressure of CO₂ (pCO₂) as this is what is required to properly equilibrate against the bicarbonate content of the medium.

- Use good quality pre-mixed gas. Good suppliers offer a “primary standard” grade in which the stated components have tolerances of ±2% relative, and each tank comes with a Certificate of Assay.

- Be careful about the gas lines – don’t use tubing that is permeable to CO₂ (or O₂) such as PVC, silicone or, Tygon®. Stainless steel piping or PTFE tubing are recommended.

- Include a VOC filter in the gas supply line.

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### General Considerations

1) **Education / Training:** Like any piece of lab equipment, read the manufacturer’s instructions for Use / Operating Manual carefully and in its entirety. It is the user’s responsibility to know how to use and maintain any and every piece of equipment in the lab. All staff should be fully trained in how to use each piece of equipment properly (e.g. changing humidification bottles).

2) **Alarms** on equipment are there to act as **warnings** that something is outside its normal operational range – this is basic engineering. Deciding whether something is a significant operational problem must be the responsibility of the user, based on each user’s particular requirements and expectations.

All operating parameters for a piece of equipment have a set point (the ideal point at which it will operate) and a pre-defined range or “span” of values that are acceptable for normal operation. An “alarm” will usually be triggered if a parameter exceeds its range for more than a certain amount of time. An alarm does not necessarily mean that the equipment has malfunctioned – just that it needs your attention. Each lab should decide what extreme values (which must be clearly outside the normal operating range) constitute a real malfunction, i.e. one that will necessitate action (such as a Non-Conformity Report), and which might compromise patient care.

A commonly seen gas flow alarm issue with BT37s occurs when the flow rate is just a few ml/min outside the set range. But unless the flow rate is substantially below the required range for a prolonged period this has no significant impact on the embryos – although it is annoying. Such “alarm” conditions are more frequent when many incubators are connected to the same gas supply, if the pipe carrying the gas to the lab is too small, or if the supply pressure is set at the lower end of the operational range. Widening the span or (better) requiring that the “abnormal” flow rate continue for a longer period before triggering an alarm, is a simple fix that would not adversely affect the incubator’s real world operation.

3) **Control sensitivity:** Given their size and nature, temperature measurements made by benchtop incubators are very sensitive. This can result in more “alarms” than one might be used to with big box incubators, whose measurement of, and response to changes in, the internal conditions are inherently less sensitive. This means that the extent and duration of fluctuations accepted by the controllers of big box incubators are inherently greater than those tolerated by benchtop incubators: the latter are simply more sensitive and responsive, requiring more intelligent setting of range limits and programming of warning alarms.

4) **Installation:** When installing a new piece of equipment – which has presumably been selected based on its design, performance characteristics, and track record of use for the intended purpose – it is expected that an **Installation Qualification** will be performed. For a new benchtop incubator this could involve:

- Ensure that the equipment has been properly located within the laboratory: away from direct sunlight and other electro-magnetic interference, correct spacing from walls and other equipment, avoiding cold draughts, etc.

- Train all staff on its proper use, including the adjustment of settings and alarms, what the indicators and alarms actually mean, and how they must be handled.

- Don’t over-tighten the Swagelok gas connector as it can split the fitting and cause a leak; check for leaks using soapy water.

- Confirm proper operation: e.g. run it for a period while logging critical operational parameters using **independent** sensors (i.e. don’t just rely on an internal logging function); run it overnight using a medium with phenol red; or do a test run with either mouse or “supernumerary” human embryos. This should also reveal issues such as over-sensitive alarm settings that cause spurious alarms.

**Note:** Only use sheathed PT100 thermocouples when making reference temperature measurements.

- Develop an in-house **Operational Qualification Programme** that includes regular periodic checks of operation (unnecessary if connected to a real-time monitoring system such as Planer’s Assure24seven) and check the battery at least quarterly.

5) **Changing humidification sets:** It has been suggested that the regular bottle/tubing set changes should be scheduled for when the incubator will not be needed for clinical use for at least 24 hrs afterwards, allowing the unit’s continuing proper operation to be verified.

Another novel feature of the BT37 is its bottle change mode; before the unit returns to normal operation it goes through an extended purge to ensure that the water is saturated with CO₂.

Some users have suggested running the unit overnight with dishes of medium containing phenol red in each incubator compartment to verify proper gassing. However, once everyone in the lab is well-experienced with the incubators, and a formal process for double-checking the gas tubes after changing the humidification bottle set is in place, such extra caution is probably unnecessary.

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