Incubator Architecture: Building a Comfortable Home for Your Cells

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The CO_2 incubator has been a mainstay of the tissue culture laboratory longer than the professional lifetime of most researchers. These reliable workhorses are entrusted to house not only the routine initial starting material for most cell-based experiments but often very precious experimental samples that are enabling scientists to uncover exciting new cellular breakthroughs in research. Here we discuss some of the key features to consider when selecting a new incubator for the lab.

Wet or dry solutions?

The traditional CO_2 incubator is a box surrounded by water-filled walls, and this technology still accounts for the majority of incubators found in labs today.

“Historically, one of the reasons people got water jacketed incubators [was] that the temperature uniformity side to side, top to bottom, is great,” says Mary Kay Bates, senior global cell culture specialist at Thermo Fisher Scientific. The sheer thermal mass of the water enables the incubator to hold its temperature significantly longer, and perhaps more uniformly, than a dry-jacketed incubator—a plus for any lab prone to power outages—and it also helps dampen any vibrations.

But taking the traditional approach can have its downside. Thick walls add to the incubator’s footprint and can squeeze its interior dimensions. Water-jacketed incubators can take up to a full day to achieve stable operating temperature. They are very heavy and need to be drained before being moved. They require maintenance. Some have the potential to leak. And they “are a real pain if you get contamination inside the water jacket,” says David Killilea, staff scientist at the Children’s Hospital of Oakland Research Institute. Killilea created and manages an academic core facility.

Dry-jacketed incubators have been around for a while, as well, but they were “not so stable, and there were some heating irregularities—there would be a warmer part of the incubator and a cooler part,” recalls Killilea. He says those issues had been worked out by about 2001, when he purchased four Sanyo incubators that use an air jacket and direct heat to wrap the chamber. He has recommended this incubator to many colleagues, he says, and recently purchased an additional two of the (now Panasonic-branded) updated model.

The majority of incubators being sold today use direct heat and/or air-jacket technology, says Rebecca Guarino, product manager, CO_2 incubators, at Eppendorf North America. Many, if not most, of the major CO_2 incubator vendors manufacture dry-jacketed models, and some (like Eppendorf) no longer even offer water-jacketed versions.

Keep it clean

For many users, avoiding the spread of contamination is perhaps the highest priority for an incubator (and one that vendors are keen to tout as a point of innovation). Robert Hunter, program manager of the University of Washington's Transgenic Resources Program recently purchased a pair of stacking Thermo Scientific Heracell incubators for his core. Because the core often gets cells coming in from outside sources, he says, "I was really interested in the copper-lined incubator, just to control fungus and spores and these types of things—and the high-temperature decontamination system."

Copper interiors are not all created equal. Some, like the Heracell, are 100% copper. Others, like Thermo Scientific's Forma line, come equipped with a stainless-steel interior, with the option of copper shelves and components. Others, like Panasonic's, offer copper-infused stainless steel—which Killilea says solves the problems of patina (discoloration) and copper dust coming from pure copper. Guarino notes that the U.S. Environmental Protection Agency has set standards for a surface's minimum copper percentage for it to have antimicrobial efficacy.

High-heat decontamination is the process by which the entire chamber is brought up to, and kept at, a specific temperature for a given amount of time.

This option is available only with dry-jacketed (not water-jacketed) incubators. The process may use moist heat, which typically does not exceed 95°C, or dry heat, which can reach as high as 200°C. In some models, certain components that can be damaged by heat must be removed prior to initiating the decontamination cycle.

Killilea's new incubators came with the option of a fast, low-temperature hydrogen peroxide vapor decontamination system. The built-in UV light—tucked behind the ductwork, so it doesn't directly impact the cells—then causes the vapor to decompose into water and oxygen. The UV light also acts to decontaminate air that cycles past throughout the day.

Other design features can also play a role in limiting contamination. Seamless interiors with rounded corners may limit areas where microbes can collect, for example, and HEPA filters prevent contamination from regular air flow. Positive pressure can help keep unfiltered lab air from entering the chamber, when the incubator door is opened.

Common sense and good sterile techniques are still important practices. Alison Killilea, one of the facilities supervisors at the University of California, Berkeley, says her core principally uses four very old Heraeus incubators: "I think they were bought in the '80s or early '90s." She comments that the incubators have no water pan. "You actually pour the water directly into the incubator, because the whole thing is copper lined." Although there is no decontamination cycle, the lab experiences very little contamination. "We just focus on having really good technique, so we don't have to use some of these backup-ish things," she says.

It's in the air

The ability to maintain a uniform temperature throughout the chamber, high humidity to minimize evaporation, and a consistent level of CO_2 so the culture medium maintains the proper pH, are the hallmarks of a CO_2 incubator.

However, trying to suss out the differences and benefits of different dry-jacket technologies in achieving these goals can be like playing he-said/she-said.

Although the lines have somewhat blurred, a direct heat system uses heated cables that are in contact with the chamber, often surrounded by some type of insulation, to maintain temperature. An air-jacketed system heats the air surrounding the chamber. Several manufactures offer hybrid systems, and at least one manufacturer (Caron) surrounds its chamber with a heat-retaining gel.

BMT’s direct heat system has three separate heating circuits—one for the door, one for the sides and one for the bottom of the chamber. “They’re heated at a slightly different rate, so we’re able to create natural convection,” explains Rick Ellison, the company’s business unit manager, Scientific Division. “Not only does the direct heat design allow us remove the fan, but [it] allows us to draw condensate to the bottom of the chamber where it belongs,” by creating a cooling point —all while keeping every spot of the chamber to within 0.1°C to 0.25°C of set point.

Meanwhile, Bates extols the virtues of a “circulating fan that creates a uniform environment throughout the chamber and ensures fast recovery of all conditions (CO_2, temperatureand humidity). In our Thermo ScientificTM Heracell™ VIOS CO2 incubators, our exclusive Thermo Scientific TM THRIVE TM active airflow design achieves recovery within 10 minutes or less from a standard 30-second door opening.”

In most units, water evaporating from a pan on the bottom of the chamber keeps the chamber humidified. For applications in which humidity control is crucial, some incubators monitor humidity and replenish it with vapor from an external reservoir. These latter are generally more popular in a GMP production type of environment, says Bates.

Hypoxia

As people move from culturing hardy cell lines to culturing primary cells, stem cells and even 3D cultures, it’s increasingly important to be able to control O_2-levels, as well, to better mimic hypoxia in an in vivo environment. Many vendors now offer CO_2 incubators in which hypoxic environments are created by introducing nitrogen gas. Eppendorf, for example, offers two options: 0.1% to 19%, or 1% to 19%, O_2 control.

There are also incubators that create hyperoxic conditions. “They’re less popular—a bit more of a niche application,” says Bates. “Sometimes people use them to study retinal cells or lung conditions or [to look] at reactive O_2 species.”

Xcell Biosciences' diminutive (3.7-liter) Avatar system takes atmospheric control one step further: “We’re able to mimic the range of pressure conditions found all the way from the circulation to the bone marrow to the fluids found within the brain,” notes chief scientific officer James Lim.

And in addition …

Not all equipment can handle high CO_2 and humidity. Several manufacturers market shakers specifically meant to be used in an incubator, some of which have controls external to the chamber. Eppendorf offer a benchtop CO_2 incubator with a built-in shaker. (There are also shakers on the market with built-in CO_2 control, achieving “a similar end result from the opposite direction,” notes Guarino.) Similarly, Lonza’s CytoSMART™ and Essen BioScience’s IncuCyte® live imaging systems are designed to be housed in an incubator.

These days, it’s not difficult to find CO_2 incubators of various sizes with touch-screen controls, data logging and Ethernet connectivity, out-of-parameter alarms, reversible door hinges, multiple interior glass doors (especially in O_2—controlled units), password-protected parameters and door locks, and even the ability to cool as well as heat the chamber. Whatever meets your lab’s needs, it’s probably out there. Just remember to always practice good sterile procedures, and that there is no substitute for common sense.

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