

*Microbial contamination, caused by bacteria, bacterial spores, viruses, mycetoza, yeast or other microorganisms, frequently presents a major risk in cell culture experiments. Since this contamination does not necessarily occur together with the overgrowth of the cultivated cell type, it is often detected too late. Changes in host cell morphology and even genetic changes such as chromosomal aberration and translocation, can, for instance, be caused by mycoplasma infection. In extreme cases, a single germ can destroy the work of weeks or months of intricate research effort.*

*In view of the significant progress in the area of sensitive cell culture applications, such as tissue engineering or regenerative cell and tissue therapy, the requirements for CO<sub>2</sub>-incubators have changed. Highest standards are thus applied to the perfection and reliability of the entire process chain, in which the CO<sub>2</sub>-incubator occupies a key position, since it must replicate the natural in vivo conditions for optimal cell growth as accurately as possible. Sterile conditions must be guaranteed for in vitro cell cultures throughout the entire cultivation period, since in addition to the risk of spreading contamination the life-threatening danger of infecting patients is ever present.*



#### Author Details:

Mr. Wolfgang Preter  
Product Management  
Binder GmbH  
Im Mittleren Ösch 5  
78532 Tuttlingen  
[www.hot-air-sterilization.com](http://www.hot-air-sterilization.com)

## Standards Compliant Hot Air Sterilisation at 180°C – a Reliable Method for Contamination Control in CO<sub>2</sub>-Incubators

Let us briefly look at the concepts of sterilisation, decontamination and disinfection. Sterilisation stands for the complete elimination and/or absence of viable microorganisms; disinfection is understood as the elimination or inactivation of all pathogens present, which frequently only represent a partial quantity of all the present contaminants, however. The term decontamination, on the other hand, can be used in various ways such as for the removal of biological or chemical or radioactive contamination, but it often does not allow any precise quantifiable conclusions to be made with respect to its effectiveness. The pharmacopeias basically specify autoclave sterilisation, hot air sterilisation and the use of ethylene oxide and sterile filtration as sterilisation methods. From these, only hot air sterilisation seems a feasible method suited to CO<sub>2</sub>-incubators. For effective sterilisation, the various national pharmacopeias have agreed upon using a 6-log reduction of viable microorganisms, which is equivalent to one viable microorganism in a million, i.e. 1:1,000,000 units. This corresponds to a reduction of 99.9999% min. in the number of test organisms that were initially used.

The various manufacturers of CO<sub>2</sub>-incubators have developed some very different concepts for the prevention and control of contamination; in this context, the requirement for sterility of a cell culture inside a CO<sub>2</sub>-incubator has posed significant technical challenges. In selecting a suitable method, the following critical aspects must be taken into account: the inner chamber of the incubator is suitable for periodic spray/wipe disinfection, which is the standard process to reduce the bio burden; easy to clean metal and glass surfaces (incubator interior, and the glass door which closes off the test space) that have no welding seams, and wherever possible should have no screw connections and/or elements which must be dismantled before disinfection (fan impellers, covers of air duct elements) to allow prompt cleaning and uniform wetting of all interior surfaces with disinfectant; reducing the number of interior fittings such as sliding rack systems and humidification systems to the absolute minimum technical essentials in order to minimise potential contamination of inner surfaces right from outset; prevention of condensation which could serve as breeding ground for germs in the incubator interior; and secure elimination of potential contamination by means of verifiable, effective sterilisation processes. In addition, the cell culture system used should prevent the introduction of airborne germs, some of which are present even under clean room conditions. Cell culture bottles with a 0.2 µm bacterial filter were found to be suitable for this purpose.

### ARE THERE ALTERNATIVES TO HOT AIR STERILISATION?

Hot air disinfection at temperatures between 120°C and 140°C, used at different contact and cycle times (sometimes combined with HEPA filter systems), which do not represent hot air sterilisation in accordance with the pharmacopeias. Disinfection with wet steam at 90° which has shown that more thermally resistant spores may not be safely eliminated. A combination of wet steam 95°C / 145°C hot air disinfection procedures in combination with HEPA filters, for which no studies regarding its effectiveness are available, and in which filters must be regularly replaced after the decontamination procedure. HEPA filter systems with different pore sizes, for example, 0.3 µm, which achieve particle reduction within the incubator atmosphere, but which also need regular maintenance. While a reduction of particles within an incubator atmosphere can minimise the contamination risk in open culture systems, such as when working with Petri dishes, it should be noted that high-

quality cell culture containers with integrated bacterial filters in the screw cap are available in order to block the entry of germs from the incubator atmosphere and to securely prevent any cross contamination between the individual cell culture containers. Inner chambers made of copper to release bactericidal copper ions through oxidation, which act as cytotoxins on the respiratory chain of bacterial metabolisms.

However, this method is not suitable for all types of bacteria species or bacteria and fungus spores and also not for viruses, and it thus offers only limited protection. The effectiveness of copper/stainless steel alloys and/or copper enriched stainless steel on test organisms as demonstrated in a series of experiments usually amounts to 99.847% to max. 99.998%, which therefore does not fulfill the sterility requirements. UV treatment by application of non-ozonogenic UVC radiation with a wavelength of 253.7 nm. The mutagenic effect of UV radiation has been proven, its effectiveness however depends directly on direct irradiation, since it has only limited penetration and is thus only suitable for the treatment of surfaces. The effectiveness of treating water in humidification systems in CO<sub>2</sub>-incubators has been described; however, it appears that additional UV treatment is not necessary, if the water in the water pan is regularly replaced with sterile,

distilled water. In addition, for airborne germs the germicidal effect seems neglectable since the dwelling time in the area of direct irradiation is marginal.

Quality is one of the most important factors for customers when establishing confidence in a company that produces chambers for sterilisation. This is why product safety has top priority in manufacturing, and in the Life Sciences sector it is an absolutely vital element. Increasingly higher

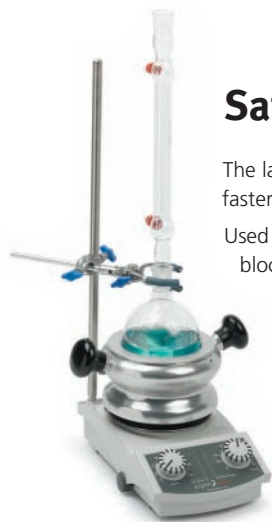
standards require researchers and

developers to have extensive know-how, and especially a sense of responsibility. With its head office in Tuttlingen, BINDER is an international leader in its industry and one of the pioneers in developing CO<sub>2</sub>-incubators with sterilisation. Its products are proven in the field, and can be found in numerous laboratories around the world.

For laboratories working in the medical sciences sector, a high-precision temperature environment is crucial for the storage and incubation of cells. The Binder CB series and C 150 CO<sub>2</sub>-incubators are especially designed for easy spray/wipe disinfection and routine auto-sterilisation. This customised design facilitates application is the ideal solution in price and handling for the customers. There are no filters or UV lamps placed inside the chamber. Therefore it is easy to clean seamless, deep-drawn inner chamber, and an integrated shelf mounting system to minimise surface contamination. Absence of condensation, even when working under conditions of highly saturated air humidity, and mechanically polished stainless steel surfaces without welding seams to prevent nesting of airborne germs. Verifiable, effective automatic hot air sterilisation at 180°C in compliance with standards, which can be performed conveniently overnight and meets international guideline requirements for hot air sterilisation. With these features the CO<sub>2</sub>-Incubators generate an optimal cell growth. This innovative technique is unique in many ways. Binder is working on the concept of hot air sterilisation to fit to the customers needs. Right now they are producing the worlds smallest CO<sub>2</sub>-incubator with 53 litre consisting all the technical details that are part of the bigger brothers with 150 or 210 litre volume.







## Safe Laboratory Heating Just Got Faster

The latest version of **Asynt's** DrySyn Classic heating blocks can heat a reaction flask 25% faster than an oil bath. Design improvements have resulted in improved heat transfer characteristics, faster heating and cooling, and even more compact dimensions.

Used in combination with a standard hotplate stirrer, these blocks conveniently replace dangerous oil baths in the chemistry laboratory. Apart from the obvious safety benefits, DrySyn heating blocks offer excellent heating performance up to 300°C, a small footprint and excellent reaction visibility, and are fully compatible with magnetic stirring. They also offer an excellent alternative to heating mantles, which are considerably more expensive (a different unit must be used for each flask size) and often exhibit uneven heating prone to 'hot spots'. The basic DrySyn Classic package consists of a base unit that takes a standard 1000ml round-bottomed flask, and a set of adaptors for other sizes, from 50ml upwards.

Asynt Managing Director Martyn Fordham, said: "The DrySyn Classic has become the standard heating device in major pharmaceutical and R&D laboratories and in many universities. Thousands of individual units and over 5000 complete systems are in use worldwide. That fully endorses the DrySyn concept, but we have a policy of continuous product improvement and this latest design now performs even better."

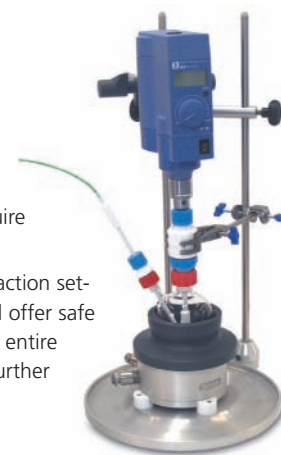
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## Affordable Controlled Heating and Cooling of Round Bottom Flasks

**Radleys** has launched Breeze, a low cost temperature control system designed to deliver rapid heating and cooling of reactions from -30°C to +165°C. Offering a safer, cleaner and more efficient alternative to using oil or water baths and heating mantles, using the Breeze in conjunction with a Heat-On block combines to deliver a new level of temperature control of reactions carried out in single round bottom flasks from 10ml to 5 litres. Combining a Breeze with standard Heat-On block and suitable thermoregulator replaces the need for separate cooling reservoir, oil bath or heating mantle, creating a compact, powerful, integrated reaction system - saving space, time and money. Benefiting from a small internal volume - Breeze is quick to respond to changes in thermofluid temperature making it ideal for solution controlled experiments such as crystallisations that require a precise temperature profile.

The integral stand and support rods facilitates simple, secure and safe setting up of Heat-On blocks and attachment of an overhead stirrer, creating a neat alternative to small reaction set-ups. The integral stand also acts as a drip tray for any condensation that may occur when the system is used at sub-ambient temperatures. Heat-On blocks are easy to set-up and offer safe temperature control of flask contents providing significant improvements in productivity. Manufactured from solid aluminium, Heat-On blocks provide even heat transfer to the entire flask, preventing hot spots. Heat-On blocks feature a unique deep well shape proven to avoid sticking and cracking of flasks and also maximising the heating surface area further contributing to faster heat-up. Heat-On blocks also feature a fluoropolymer coating providing excellent chemical resistance.

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ADVERTORIAL

## New Incubator CO<sub>2</sub> Analyser Meets Researched user Demands - Gives Accurate and Independent CO<sub>2</sub> Verification and is Easy to Use and Read

From independent research among users, Geotech has designed its new G100 incubator CO<sub>2</sub> analyser. The G100 is an easy-to-use, handheld CO<sub>2</sub> analyser with increased accuracy in the 0-20 percent range and a bright, easily read display. Readings are stable thanks to inbuilt moisture removal. Timesaving twin-probes check incubator top and bottom temperatures and difference simultaneously. Increased memory holds 1000 readings. Options include O<sub>2</sub> and/or RH analysis, temperature probes, data logging, and USB-link software to download data, analyse and export it - and to enable Internet file exchange with Geotech technical and customer support. The fully functional G100 is priced from £1000 (+VAT in UK) and is available from: [www.geotech.co.uk](http://www.geotech.co.uk)

Research has ensured the G100 analyser is fully operable and configurable from its keypad and screen. Routine, back-to-factory service and re-calibration is recommended once a year. When due, the analyser displays a time-for-service warning but continues to work normally.



Geotech's New G100 Incubator CO<sub>2</sub> Analyser (1) in its optional hard carry case to assist with protection and long operational life (2) with one of its two temperature probes for top and bottom temperatures simultaneously (3).

An optional 'USB-link cable and G100 software package' supports PC and Internet connectivity. This enables data download to a PC of the event log, user calibration and the data set for analysis, graphing, secure archiving and, if necessary, exporting to spreadsheet, word processors or e-mails. To assist users anywhere in the world, the USB-PC link and G100 software allow user settings also to be downloaded as a PC file and e-mailed to Geotech for technical support and troubleshooting. This assists remote diagnostics and service support worldwide avoiding unnecessary return to factory. It also helps ensure continued use and minimal downtime and limits time-zone lag. If necessary, the analyser firmware can be 'flashed' with a new Geotech software release, via website downloaded, for continuous improvement.

Technical specification, operational diagrams, user manual and more at: [www.geotech.co.uk](http://www.geotech.co.uk)

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## New ! G100 Incubator CO<sub>2</sub> Analyser

### Accurate and Independent CO<sub>2</sub> verification




Customer feedback asked for:

- ✓ Improved accuracy 0-20% range CO<sub>2</sub>
- ✓ Quick and easy verification of CO<sub>2</sub> levels
- ✓ Increased stability of readings by removal of sample gas moisture
- ✓ Time-saving, dual-temperature point readings, top & bottom
- ✓ Increased data storage with user-friendly, USB-PC download link & on-line support
- ✓ Easy-to-read, large, well-lit display
- ✓ Optional O<sub>2</sub>, humidity and data logging

The G100 offers all of the above for IVF, Embryology, Laboratories, Research and Medical

**Tel:** +44 (0) 1926 338 111  
**Email:** [sales@geotech.co.uk](mailto:sales@geotech.co.uk)  
**Web:** [www.geotech.co.uk](http://www.geotech.co.uk)



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