The ECACC Cryostorage Facility: Development of a cGMP Safe Depository

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The Need for Second Site cGMP Storage

Cultured mammalian cells, often following genetic manipulation, are increasingly associated in some way with the manufacture and/or testing of a therapeutic substance that is manufactured according to cGMP. The current direction of EU regulations is towards extending the requirements of cGMP further "upstream" of the manufacturing process, to include clinical trial materials and the early provenance of cell lines used in a cGMP operation. Consequently any cell line that is likely to be used in a cGMP process should be banked as a Master Cell Bank (MCB), and validated early in the research and development phases of a project.

Subsequently, all the work conducted using cells from this MCB represents a cumulative investment, which eventually can assume a very high value. In order to secure such an investment the owners of the MCB are advised to arrange a second site, back-up storage for either a part of the original MCB or a direct derivative. Cells recovered from back-up storage must be fit for use in the same cGMP operation as the Primary stock so the second site storage conditions need to be cGMP compliant.

For many years ECACC has provided second site storage for valuable third party cell lines. In recognition of the increasing need for a Safe Deposit facility that is able to support the needs of cGMP operations, ECACC has set up, and is now validating a dedicated cGMP Safe Deposit facility.

The ECACC Safe Deposit Facility for cGMP Cell Banks

The cGMP Safe Deposit vessel is being established within ECACC's new, state-of-the-art cryostorage facility located on the Health Protection Agency Porton Down site. This facility is serviced by two 10,000L liquid nitrogen tanks providing >100% reserve capacity. The Inventory storage vessels are configured for automatic replenishment and each vessel is monitored by an electronic telemetry alarm system. This facility benefits from the latest safety advances including oxygen sensors linked to powerful air ventilation. A 24 hour security service operates at the Porton Down site.

The cGMP Safe Deposit vessel is a Custom Biogenic Systems V3000 Isothermal model (Figure 1) designed so

Figure 1. Validation of cGMP Safe Deposit vessel.

that the liquid nitrogen is contained in a "jacket" which surrounds the storage compartment. This design allows vapour phase storage, which minimises the opportunity for cross contamination between vials through the liquid nitrogen medium. In addition the temperature distribution within an Isothermal vessel covers a narrower range, and can be more closely controlled when compared to a conventional vapour phase vessel.

The "conventional" design of a liquid nitrogen storage vessel requires that the liquid nitrogen is delivered into the actual storage compartment of the vessel. The inventory is then stored either submerged in a large volume of liquid nitrogen (liquid phase storage), or otherwise in the gaseous space above the surface of a smaller volume of liquid nitrogen (vapour phase storage). Liquid phase storage guarantees a constant storage temperature of –196^{0}C, but presents a number of operational and biological risks the most outstanding of which is the possibility that contaminants may pass between vials through the liquid nitrogen medium. Such a mode of contamination was demonstrated when hepatitis B virus contaminated units of human bone marrow stored in liquid nitrogen at a UK blood laboratory. Consequently vapour phase storage is considered more suitable for a cGMP Safe Depository that will contain multiple cell lines.

In a conventional liquid nitrogen storage vessel, vapour phase storage has the disadvantage that a temperature gradient inevitably extends from the surface of the liquid nitrogen to the upper regions of the compartment just beneath the lid. This temperature gradient expands and contracts as the liquid nitrogen fill level fluctuates. Provided the temperature does not exceed -135^{0}C, such fluctuations are unlikely to affect the cell stocks. This value represents the glass transition temperature of water below which molecular movement ceases and all biological activity is suspended. Nevertheless broad, fluctuating temperature gradients make it more difficult to control this critical threshold and therefore should be

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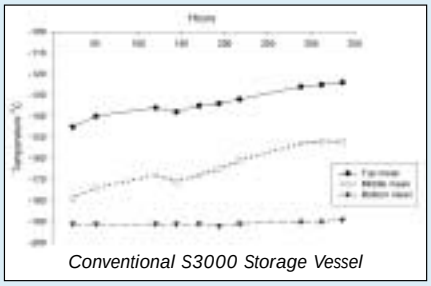
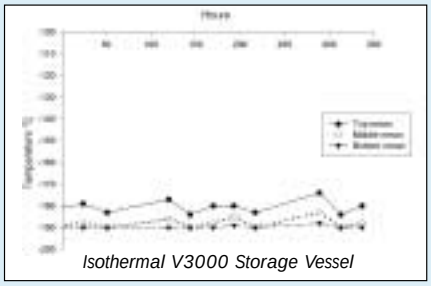
(i) Temperature Profile at Different storage levels within the vessels

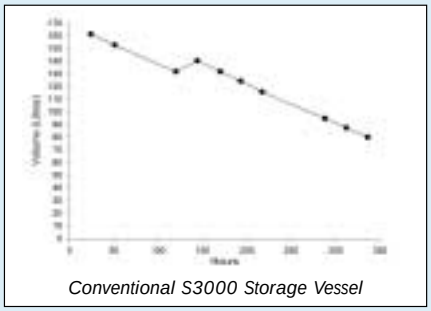


Conventional S3000 Storage Vessel

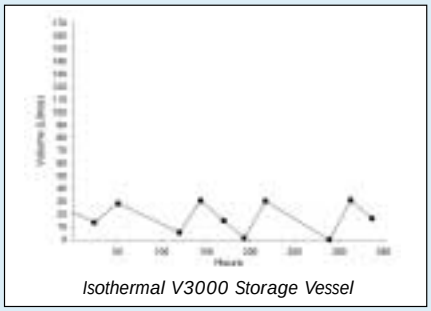


Isothermal V3000 Storage Vessel

(ii) Decrease in Liquid Nitrogen Volume over time



Conventional S3000 Storage Vessel



Isothermal V3000 Storage Vessel

Figure 2. *A comparison between the Conventional S3000 and Isothermal V3000 Storage Vessel. At the start of the experiment the temperature at the top of the Conventional S3000 storage vessel is approximately 40°C warmer than the bottom of the vessel compared to 10°C for the Isothermal V3000 storage vessel (i). As the liquid nitrogen volume decreases (ii) the corresponding temperatures at the top and middle of both vessels increase. This increase is significantly lower for the isothermal V3000 vessel in that the temperature remains below –170°C at all times. However, the temperature in the conventional S3000 vessel continues to decrease to below –130°C for the top of the tank which is higher than the critical glass transition temperature of –135°C. Therefore the isothermal V3000 tank provides a more stable storage environment than the conventional S3000, which is highly dependent on the volume of nitrogen in the tank, to achieve the required temperatures particularly towards the top of the tank.*

minimised. Isothermal inventory storage vessels have been identified as a means of achieving this.

Validation of the cGMP Safe Deposit Isothermal Vessel

The cGMP Isothermal storage vessel has been temperature mapped to determine the temperature at different locations in the storage compartment. These determinations have been repeated at different points in the liquid nitrogen fill cycle. The effects of opening the lid and the subsequent recovery times have also been determined. Similar mapping has been applied to a conventional vessel for the purpose of comparison. Results are summarised in Figures 2.

Operation of the ECACC cGMP Safe Depository

Only cell stocks that have been tested and shown to be free of microbial contaminants will be stored in the cGMP Safe Depository. In particular candidate Safe Deposits must be thoroughly tested for mycoplasma contamination. It is more difficult, perhaps impracticable to eliminate the possibility of any virus contaminant, which is why vapour phase storage is so important.

Features of the ECACC cGMP Safe Depository:

- The operational procedures for Isothermal V3000 Storage Vessel have been validated
- Vapour phase storage to minimise the opportunity of cross contamination
- Security and restricted access
- Confidentiality
- Staff on site 24 hours each day
- Maintenance and supervision by specialist, trained staff
- Eurotherm Chessel telemetry alarm system
- Continuous temperature logging, both electronic and manual
- Regular resuscitation of standard control “Monitor” cell lines that are stored in the Safe Deposit vessels
- Event logging and reporting
- Annual reporting of stock status, stock movements, the maintenance of storage conditions and the results from monitor cell lines

Conclusion

ECACC has been storing cell banks for almost 20 years and has one of the largest liquid nitrogen repositories for animal cells, in Europe. Recent initiatives will enable ECACC to make this expertise available to those who wish to secure cGMP cell stocks. For further details, including prices visit the ECACC website on **ecacc.org.uk**

Reference

Tedder, M. Zukerman, A. Goldstone, A. Hawkins, A. Fielding, E. Briggs, D. Irwin, S. Blair, A. Gorman, K. Patterson, D. Linch, J. Heptonstall and N. Brink. 1995. Hepatitis B transmission from contaminated Cryopreservation tank. Lancet 346:137-140

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