The maintenance of precious biological samples in a frozen state is the well-established, proven and reliable method for the storage and archiving of biological samples, and a large amount of effort is devoted to validating storage conditions and monitoring cold chain conditions to verify that, once frozen, the samples remain below a specified temperature. The handling of the sample prior to its freezing and the nature of the freezing process itself is however in general much less controlled than the subsequent custody of the sample in its frozen state. As a result, a world wide effort is being undertaken by various bodies to develop new standards for the appropriate collecting, handling and processing of blood, tissue, tumour and other biological specimens (e.g. the European Union www.devicelink.com/ivdt/archive/09/04/003.html; or the United States National Institutes of Health, http://biospecimens.cancer.gov/practises/default.asp).

Currently there are several methods that are usually used in labs for the freezing of samples.

Liquid nitrogen and solid carbon dioxide

With its boiling point of -196 °C, liquid nitrogen is in theory one of the optimal methods of rapidly and reproducibly freezing biological samples. In practice, however the technical installations and infrastructure necessary to provide a ready source of liquid nitrogen mean that this method is of limited overall application.

Solid carbon dioxide (dry ice) is much more widely available; its sublimation point is -78° C. However several practical problems arise in its use.

Solid CO₂

Dry ice is generally provided in slabs, or in powdered, pelleted form. In all cases the condensation of water vapour from the atmosphere quickly results in the formation of a "skin” of ice on all exposed surfaces of the dry ice. This ice layer not only acts as a thermal insulator between the cold dry ice and the tube containing the sample, but in addition...
the ice skin bonds the previously granular pile of solid CO₂ into a solid mass, making it difficult to introduce new tubes without boring new holes to accommodate them. As if this weren’t enough another problem arises from the sublimation of the solid CO₂ as a result of its initial contact with the relatively warm sample-containing tube. The resulting CO₂ gas also acts as an insulating layer preventing thermal transfer between the dry ice and the sample. All this means that the kinetics of the freezing process can vary significantly from one tube to another, with consequent variable (and detrimental) effects on the properties and characteristics of the biological sample.

Solid CO₂ in slurry form
The above disadvantages of solid CO₂ are generally overcome by suspending it in a suitable solvent such as alcohol, which has the advantages of holding the temperature at the -78°C of the sublimation point and of avoiding the creation of ice skins or gaseous CO₂ insulating layers so greatly increasing the freezing rate and reducing variability. As all biological scientists know to their cost, however, the price for this is the danger that identification labelling such as “indelible” markings on the tube are removed. Even expensive and supposedly solvent-resistant labelling systems have a heart-breaking tendency to “float” away in the CO₂/alcohol slurry.

Thermoconductive sample tube holders
A solution to the above apparently trivial yet potentially serious problem from the point of view of reproducibility of ultimate data has been developed by the BioCision company with the introduction of their range of thermoconductive tube holding devices, such as the Coolrack device [Figure 1]. Due to the thermoconductive properties of the alloy from which they are constructed, the sample racks not only adjust rapidly to the cryogenic temperature during set-up, but efficiently transmit the heat from the sample to the dry ice, so providing freezing rates that are equal to, or greater than, those that can be achieved by placing the sample directly in the dry ice. By removing the need for the creation of an individual recess in the dry ice for each tube, the racks provide an easy and regular template for the storing of each tube so that they can be readily identified, which is not always the case in samples stored willy-nilly on ice or dry ice granules [Figure 2]. In addition, the spacing of the wells in the rack allows for a much higher density in the sample array, with the result that there is a significantly reduced overall footprint for the freezing system.

However, the greatest advantage of the new system is that the increased uniformity and standardised performance provided by the rigid control of the physical parameters of the freezing process can be embedded into the sample collection protocol. As the operator-dependent variables in sample collection are reduced or eliminated, protocol compliance becomes almost the default situation. The freezing profiles associated with thermoconductive rack freezing methods are highly repeatable and can thus be eliminated as a potential source of error [Figure 3]. The absence of sample collection variance can be of particular benefit in multiple-site clinical trials or large collaborative research studies where the influence of operator-dependent variance is compounded.

BioCision
Larkspur, CA, USA.
www.biotech-online.com & search 12566

Figure 1. The random layout of tubes stored in classical ice or dry ice baths can lead to a loss of time as each tube is individually identified or, even worse, can result in errors where a reagent is added to the wrong tube.

Figure 3. Just as important as the rate at which biological samples are frozen is the reproducibility of the freezing process itself. In the above experiment repetitive freeze/thaw cycles were carried out using cryo vials loaded in CoolRack blocks with each vial containing 1.8mL water and fitted with a thermocouple. It can be seen that each sample goes through the 0°C transition in an identical way. The apparently homogenous temperature plots are in fact the composite of five separate data plots, which are essentially superimposable.