Cryogenic Storage of Biologic Materials in Screw Capped Vials

There is a growing body of literature from research and government laboratories concerning the wisdom and safety of storing biological materials in liquid nitrogen (cryogenic) freezers, both in liquid and vapor phases. This is a general, opinionated, discussion of some of the considerations for very-deep freeze sample storage.

THE NEED: From my own experimental work, and from a legion of others as well, cryogenic storage of biological samples is both desirable and necessary. The so-called "glass temperature" of -132°C is the temperature at which biological activity ceases. While mechanical devices do exist that maintain temperatures below (barely) this glass temperature they are noisy, heat generating devices that require liquid nitrogen backup anyway to avert disaster should the electricity be disrupted or the device fail. This makes simple liquid nitrogen storage at -196°C a very popular choice for storage in many labs.

THE ISSUES: There seems to be universal consensus now that liquid nitrogen can be a transmitter of biological contamination between samples. To make matters harder on the scientist wishing to safeguard samples in storage, a recent article told of the vapor phase spread of aspergillus inside a freezer between samples stored in screw capped vials. Clearly, there is always the problem of inadequate aseptic technique when handling the vials, but that is the point of another discussion. This also shows a need to keep clean freezers, but this is a practice I find to universally absent from lab protocols.

Besides the integrity of the sample, there is the issue of **exploding vials** that have been stored immersed in liquid nitrogen. Under the condition that liquid nitrogen had leaked into a vial in storage, that vial is very possibly going to explode when taken into the lab environment due to the rapid boil off of the trapped nitrogen. This explosion raises two ugly problems for lab personnel; the physical danger from shrapnel and the biological danger from the splattered biologicals in storage.

Screw Cap Vial Leakage: The vial manufacturers have become quite aware of the potential liability they face should they recommend their vials be immersed into liquid nitrogen. Under the proper conditions the vial may be perfectly capable of immersion without leakage, but the manufacturer has no control over the handling of the cap/vial connection, which is clearly the point of contention. Therefore, to protect themselves all vial manufacturers now demand that their vials only be used in vapor phase cryogenic storage.

Why do the vials leak? Cryogenic temperatures are certainly hard on materials, and the vial plastic and washer materials become brittle to varying degrees, depending on the material. It looks easy to blame the vial manufacturer for having poor molds or QC procedures. The real culprit for everybody is differential pressure that develops when the vial is cooled 200°C. Using the thermodynamic equation $pV=nRt$ (p=pressure, V =volume, n= moles of gas, R is a constant, and t=temperature in kelvin degrees) we can see the situation. In a given vial, the volume remains the same as does n and R. When the kelvin temperature is reduced in liquid nitrogen to 28% that of an ice bath temperature, the pressure inside the vial is reduced by the same proportion. That means there is about a 10-psia difference in pressure forcing liquid

nitrogen into the vial. If the cap were not screwed on exactly properly, without either under-torquing or over-torquing the cap in place, then it's likely the cap will admit liquid. Of course, in the vapor phase this may also allow air-borne pathogens to be admitted to the vial as well.

Protect Yourself and Your Samples: There are a few steps a scientist can take to reduce the likelihood of contamination due to leaking vials:

- Avoid altogether immersing sample vials in liquid nitrogen. This may mean reconfiguring or replacing old style freezers.
- Choose and use vials carefully. Always choose vials with caps made of the same plastic in order to avoid differential contraction that could lead to loose caps. Also, use vials with female caps having a high quality gasket that is unable to deform easily, and tighten securely without over tightening. This avoids the opportunity of over-torque with "squishing out" of the gasket forming a leak path (*The Simport T308 model or the T-310 model CryoVials® are excellent examples).*
- Use cryogenic freezers specifically designed as vapor phase storage systems that have a slight positive pressure in the sample chamber which circulates out pathogens (and water vapor that causes the clouds that you can't see through in old style freezers). *The Custom Biogenic Systems' patented Isothermal Freezer is an excellent example of this type of freezer.*

This discussion of choosing vials for their design is not nearly so important when the storage is at ultra low temperatures (-85°C) or warmer because the pressure calculation is very much reduced.

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