Liquid Nitrogen Consumption in Cryogenic Freezers

As more research workers realize the desirability of storing biologicals in cryogenic temperatures, larger, dry freezers are newly appearing. It is my experience that the physics and mechanics of refrigeration are not high on the list of things that interest bio-researchers until something appears to be wrong. It is not a frequent event that anything is actually "wrong" with a large vapor phase liquid nitrogen freezer other than they are different from the smaller Dewar-style freezers that have traditionally been in labs.

Why are these new vapor-phase freezers appearing in such numbers recently? There are at least 3 reasons I have found for this proliferation:

1. Lab floor space is ever harder to come by, and multiple small freezers are being consolidated into larger central freezers. This is a more efficient use of valuable space.
2. Biologicals can continue to break down in the warmer temperatures of –80℃ ultralow and –20℃ standard freezers. When in doubt, use cryogenic storage to insure stability.
3. Old style, in-liquid freezers have conclusively been shown to be vectors of contamination transfer between samples. While it is possible to hold samples in vapor using Dewar-style freezers, it is far more difficult to manage the liquid level to avoid accidental liquid submersion.

{Q} So, what is there to complain about with these large freezers that can hold tens of thousands of samples?
{A} They use more nitrogen than small Dewar-style freezers, even though they are working as designed and anticipated.

Well, why is this expected?

Simply, the extra nitrogen is lost because of the size of the freezer and the convenience of access to the inventory. Most of the "**Static**, or **Inherent**", **Nitrogen Loss** in any cryogenic freezer is through the top (see the index piece from the folks at www.howstuffworks.com for the construction and characteristics of Thermos). Traditionally, the Dewar-style freezers had very small openings (2" or 2.5") and top plugs to minimize heat gain. This design, however, severely limits the number of samples contained because the rack has to hang from the lip and be able to fit through the opening. Currently, the upper capacity for these Dewar-style freezers is around 6000 x 2 ml samples with a 8-1/2" neck opening. This freezer has a top opening large enough to fit racks holding 10 standard 2" boxes that can hold 100 samples each. The best fit has only 6 racks.

Once you want more racks in the freezer the top gets larger and more heat enters (to burn off liquid nitrogen). When you want more racks in the freezer it becomes a better design to have the racks sitting on the bottom. If the racks sit on the bottom the opening has to be large enough to easily access them for removal. You can see where this goes; the opening gets bigger as the capacity increases and the static losses increase.

Unfortunately, there is more. As more people use the freezer even more nitrogen is lost because the top is opened and samples added/removed. This is the **"In-Use" Nitrogen Loss** that cannot be calculated prior to operating the freezer in any given lab situation.

Then there is the **"Fill** or **Transfer" Nitrogen Loss** that must occur every time the freezer is refilled with liquid nitrogen from a source tank. All that hardware between the interior of the source tank and the interior of the freezer will boil away nitrogen until it is cooled down from room temperature (+21℃) to liquid nitrogen temperature (-196℃).

Remember, also, that **the source tank also has a static loss** of some 1.5 - 6 liters per day just sitting there. And that 180-liter source tank you paid for; well, it probably didn't arrive with 180 liters. More likely, it had less because of the way the supplier fills, stores, and transports it.[1]

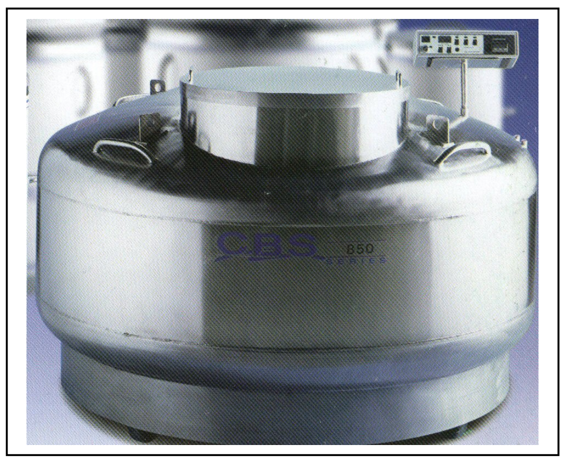
These 4 types of losses are common to all freezers whether large or small. It is just a characteristic that the larger the freezer the larger the losses. So let's do a calculation for a CBS V-3000 Isothermal Freezer that can hold more than 22,000 x 2ml sample vials.

1. Static loss of 9 liters per day
2. Use loss; it's up to you, 0-8 liters per day maybe
3. Transfer loss of 3-6 liters per event (try to make the hose as short as possible and only fill when really needed, such as every other day)
4. Source cylinder static loss of 1.5-6 liters per day (generally, better on new equipment, worse on old)

This works out to 12 liters, and up to 29 liters, per day depending on usage and condition of equipment, (or 6 days of use from the source tank for worst conditions to about 15 days at best with, reasonable, 12-14 days expected).

Cryogenic Freezer Design Options

These freezers are both from Custom Biogenic Systems.



Why would you prefer one to the other? Both have microcircuit control of liquid level and alarms. The big beast on the right (model 850) actually has about twice the capacity for 2 ml vials that the V-3000 has (larger of the 2 in the left picture) and it consumes less liquid nitrogen by static loss.

The model 850 is an old style freezer that has liquid nitrogen in the sample chamber. Originally built to be filled with nitrogen for liquid phase storage, to run it as a vapor phase unit there has to be *at least* 6 inches of box storage lost at the bottom for the liquid coolant (this is a common trait for all freezers other than the CBS Isothermal line). Also, access to the samples is through a round hatch. Retrieving the racks means that there has to be a carousel that can be rotated to bring the racks under the opening. Retrieval of some of the

[1] I know that this is an incredibly confrontational thing to say, but the freezer manufacturer has done numerous tests to prove it. The gas suppliers will adamantly deny this, and they know you probably have no way of checking them on it. The only acceptable way to prove whether that the tank is actually filled is to weigh it before and after a fill; a very inconvenient procedure for just about every customer. One liter of liquid nitrogen weighs 1.78 pounds, so a 180-liter fill adds 320 pounds to the tank that weighs about 250 pounds.

racks may require removal of other racks that are blocking. There are certainly times when removing racks while finding others can put samples in danger of warming sufficiently to be harmful to the product. Since there is a considerable distance from the liquid nitrogen to the top of the freezer there can be a significant temperature gradient from the cold low level to the warmer upper level. And then, there is the problem of the cloud in the chamber that obscures your view that is a considerable problem in these enclosed freezers (blowing on the cloud makes it worse BTW).

The V-3000 Isothermal model is the CBS patented design that is designed as a vapor phase freezer from inception. The liquid nitrogen is housed in a jacket around the sample chamber that keeps the upper level samples about the same temperature as the bottom ("isothermal" top to bottom). This means zero contact of samples and liquid and no loss of sample storage space. The full access top allows immediate retrieval of any rack in the freezer without removal of any other. There is a very thin cloud at the top that is very easily blown off by partial fill.

So, you see the real comparison of the freezers; the Isothermal does consume more liquid nitrogen than the model 850 because it keeps the samples colder and has a much larger top area. The choice becomes how *you* weigh the factors of ease of access and safety for biological sample product as opposed to higher liquid nitrogen consumption when you have large numbers of samples to store.

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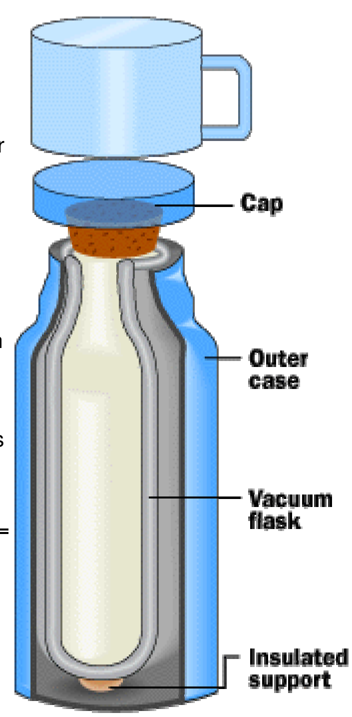
Inner Workings of a Thermos

(from www.howstuffworks.com)

One way to build a thermos-like container would be to take a jar and wrap it in, for example, foam insulation. **Insulation** works by two principles. First, the **plastic** in the foam is not a very good heat conductor. Second, the **air** trapped in the foam is an even worse heat conductor. So conduction has been reduced. Because the air is broken into tiny bubbles, the other thing foam insulation does is largely eliminate convection inside the foam. Heat transfer through foam is therefore pretty small.

It turns out that there is an even better insulator than foam: a **vacuum**. A vacuum is a lack of atoms. A "perfect vacuum" contains zero atoms. It is nearly impossible to create a perfect vacuum, but you can get close. Without atoms you eliminate conduction and convection completely.
What you find in a thermos is a **glass envelope** holding a vacuum. Inside a thermos is glass, and around the glass is a vacuum. The glass envelope is fragile, so it is encased in a plastic or metal case. In many thermoses you can actually unscrew and remove this glass envelope.



A thermos then goes one step further. The glass is **silvered** (like a mirror) to reduce infrared radiation. The combination of a vacuum and the silvering greatly reduces heat transfer by convection, conduction and radiation.

So why do hot things in a thermos *ever* cool down? You can see in the figure two paths for heat transfer. The big one is the **cap**. The other one is the **glass**, which provides a conduction path at the top of the flask where the inner and outer walls meet. Although heat transfer through these paths is small, it is not zero.

Does the thermos know whether the fluid inside it is hot or cold? No. All the thermos is doing is limiting heat transfer through the walls of the thermos. That lets the fluid inside the thermos keep its temperature nearly constant for a long period of time (whether the temperature is hot or cold).

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