Sample Integrity Focus

How safe are your samples?

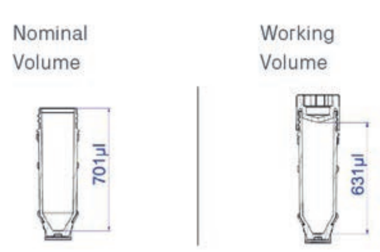
Part II – Working Volume

Alexis MacLeod, Azenta Life Sciences, alexis.macleod@azenta.com

Plastic storage tubes are used in laboratories worldwide to store biological and chemical samples across a wide range of temperatures from ambient to -196°C. Knowledge of the working volume is a key criterion to consider when selecting the most appropriate tube and, is dependent on a range of factors including:

- Fill volume of the tube
- Accuracy of the volume dispensed
- Freezing conditions
- Thawing conditions
- Cap selected
- Burst pressure of the cap

Manufacturers of sample storage tubes specify the size of tubes in a variety of ways, but rarely state the sample type or storage conditions required to achieve the volumes stated. This can lead to catastrophic results if the working volume of the tube is exceeded.



Manufacturers use nomenclature such as:

- Tube size
- Nominal volume
- Working volume
- Safe storage volume

Figure 1: Nominal volume vs working volume.

Fill volume

The impact of overfilling is more profound for biological samples with a high water content, which, due to its unique properties, creates challenges when storing and freezing samples.

When water freezes, the molecules form a crystalline structure maintained by hydrogen bonds which push the molecules further apart. As a result, solidified water, i.e. ice, is less dense than the liquid format, which is why ice cubes float in our drinks.

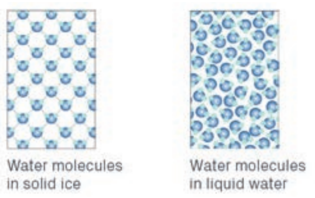


Figure 2: Water molecules vs water molecules in solid ice in liquid water.

A consequence of this is that the volume occupied by water increases by approximately 9% as it freezers. It is this expansion of the volume that can lead to cracking of sample storage tubes if the working volume is exceeded, with a resultant loss of precious samples.

As we can see from *Figure 3*, the lowest density and therefore the largest expansion of water (and by association biological samples), occurs at the freezing point (0°C for pure water and slightly lower for biologicals).

The impact of Charles Law on sample volume

Charles' Law states that the volume of a fixed mass of gas is directly proportional to the temperature at a constant pressure. As the temperature of the sample decreases, the air filling the space between the sample and the cap (headspace) will also decrease at a linear rate at temperatures between 20°C to -80°C.

Even if the extent of the 'Over Fill' is not enough to crack the tube, the expansion of the sample elevates the pressure in the tube which can lead to the cap or O ring 'popping out' (*Figure 4*). What happens when an internally threaded tube is overfilled by only 5% - increased pressure in the tube causes the 'O' ring to pop out with loss of tube integrity.

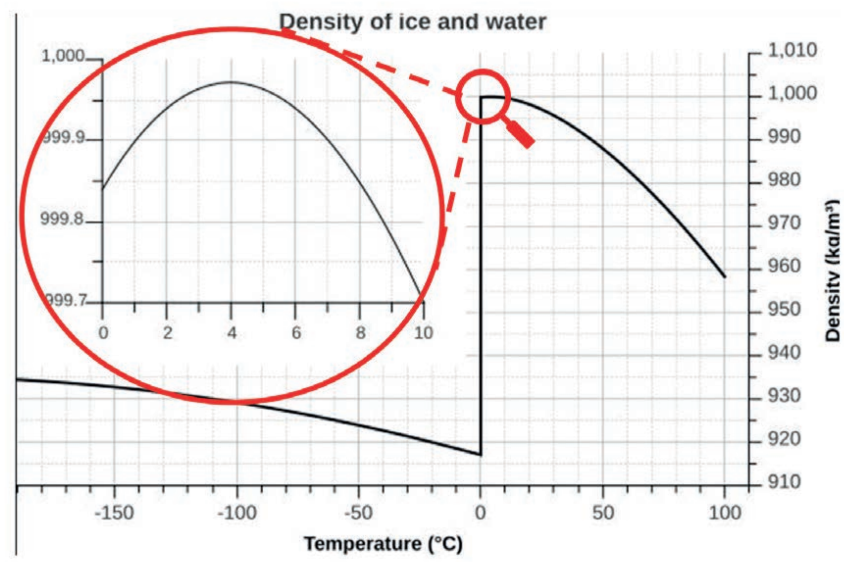


Figure 3: The lowest density and therefore the largest expansion of water occurs at the freezing point.

To address this issue, Azenta Life Sciences exploited the co-moulding technique to weld an integrated TPE-gasket to the polypropylene cap, in place of a traditional silicone 'O' ring, eliminating the potential loss of sample integrity associated with expulsion of the O ring.

Azenta Life Sciences was also the first to develop the External Thread 96 format sample storage tube, which has the benefit of maximising the internal volume and therefore working volume for a given height of tube. Unlike the traditional internal thread tube, additional sample volume can be accommodated as the cap does not ingress into the tube.

Calculating the safe working volume

The safe working / storage volume of a tube can be calculated to ensure the internal pressure generated during freezing does not exceed the burst pressure of the sealing method.

2Bar pressure should be considered the maximum safe pressure even if the seal is capable of a higher pressure in order not to damage the tube itself.

Figure 4

$$V_2 = \left(\frac{V_1}{T_1}\right) \times T_2$$

V1 is the original volume of the gas
V2 is the new volume of the gas
T1 is the original temperature of the gas (Kelvin)
T2 is the original temperature of the gas (Kelvin)

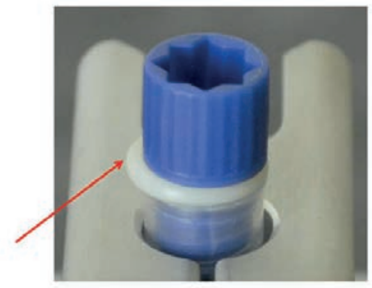


Figure 4: The expansion of the sample in the tube can lead to the cap or O ring 'popping out'.

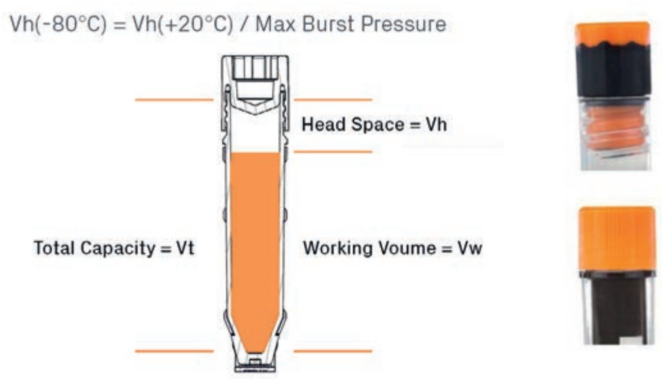
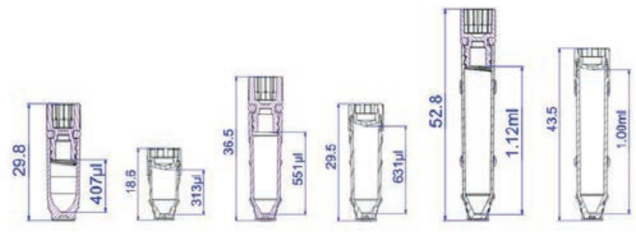


Figure 5: Calculating 'headspace'



Tube Name	0.5ml (Int)	0.3ml (Ext)	0.7ml (Int)	0.7ml (Ext)	1.3ml (Int)	1.0ml (Ext)
Nominal Volume	407ul	313ul	551ul	631ul	1120ul	1000ul
Working Volume (ambient)	366ul	282ul	496ul	631ul	1008ul	900ul
Safe Storage Volume (frozen)	340ul	260ul	460ul	525ul	933ul	833ul

Figure 6: Calculating safe working / storage volumes

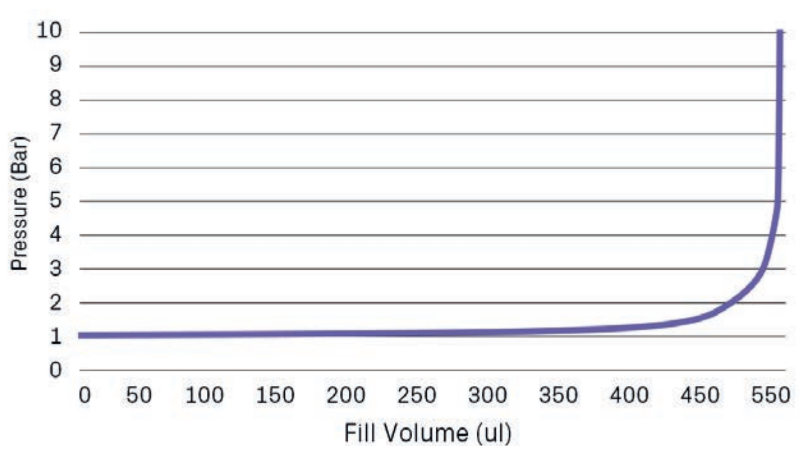


Figure 7: Impact of Charles Law on Sample Volume

As can be seen by the graph in *Figure 7*, as you get closer to the maximum safe working volume, the pressure inside the sample storage tube increases exponentially.

This is due to the ratio of sample volume to headspace. This highlights the importance of knowing the fill volume of the sample tube, which also requires knowledge of both the accuracy and precision of the liquid handling system being used.

Figure 8: Fill error of 5% leads to the tube cracking

The maximum fill volume should be to set to the maximum safe storage volume for the tubes minus the % accuracy and the % precision of the liquid handling system being used.

Using the example below for the 0.7ml External Thread tube, the calculated safe working volume is 525ul. This generates a maximum internal pressure of 2Bar, however due to the large sample volume compared to the low headspace volume, only a small error in the target fill volume can produce a significant increase in pressure. A fill error of just 5% will result in an internal pressure of over 3Bar and will likely lead to the tube cracking or the push cap popping off.

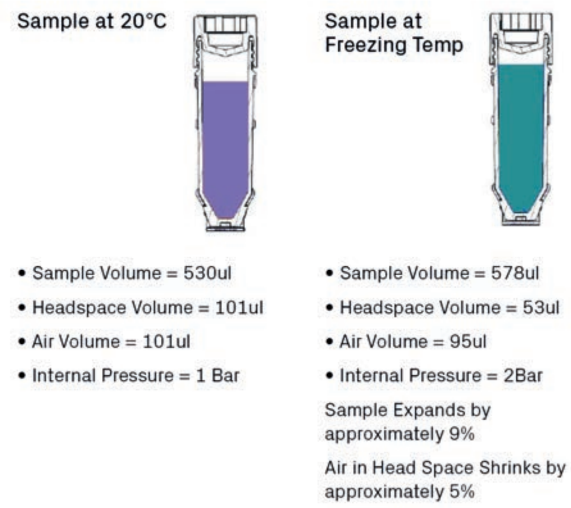


Figure 9: Sample at 20C v Sample at freezing temperature

Controlled rate freezing

Another important consideration is the method of freezing. Ideally the sample should be frozen in a controlled rate method, to ensure that the sample is frozen from the bottom upwards allowing it to expand when freezing. If the sample freezes too rapidly, it can become super cooled before nucleation occurs and the phase change from liquid to solid (ice) takes place.

If this happens an ice plug can form at the top of the sample, limiting the space available for the rest of the sample to expand as it freezes. As the sample cannot expand vertically due to the ice plug, the resulting force is horizontal causing the polypropylene to crack.

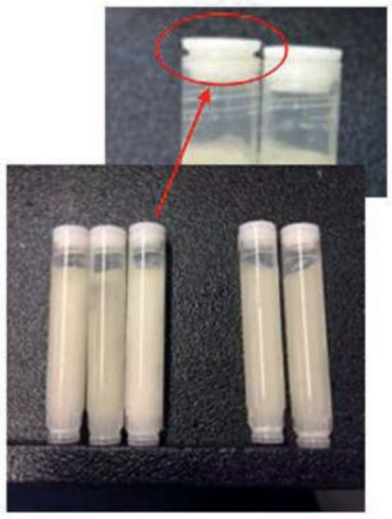


Figure 10: Example of TPE cap popping during thawing

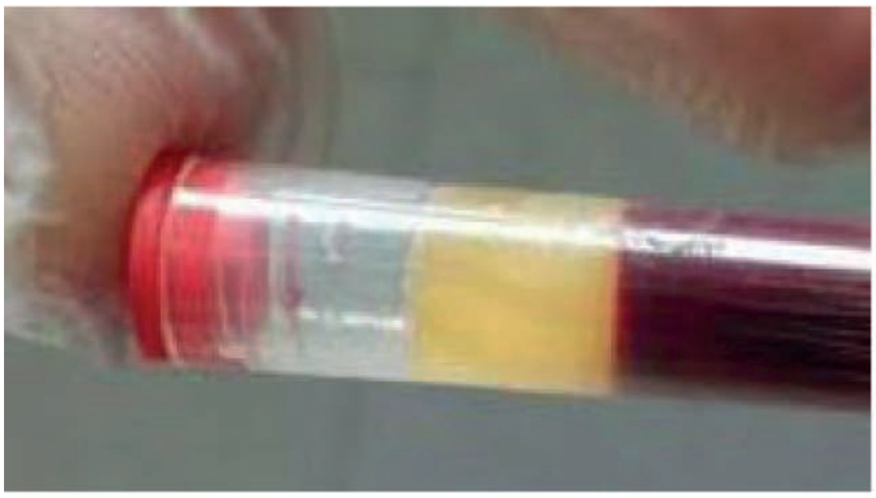


Figure 11: Ice plug forming at the top of the sample, leading to tube damage

Azenta Life Sciences offers a range of products that enable controlled rate freezing. Products are identified in the following posters: 'Best Practices for introducing -80°C Bio-Samples into -190°C Vapour Phase Liquid Nitrogen Freezers - with Experimental Evidence of Thermal Excursions' and 'Temperature Controlled Manipulation and Alcohol-free Cryopreservation: A new era in Sample Handling and Biobanking'.

Figure 12: Hinged Cryoboxes from Azenta Life Sciences

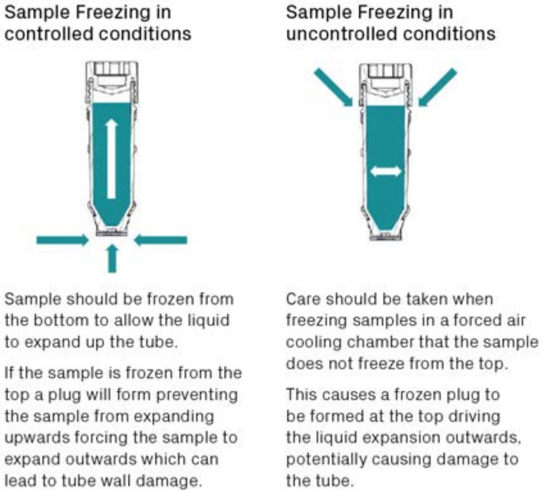


Figure 13: Sample freezing in controlled and uncontrolled conditions

Conclusion

This evaluation shows that a comprehensive knowledge of working volumes is needed to protect the sample tube, and therefore the sample, from damage which could lead to sample loss. This understanding should be based on several key factors including; calculation of potential overfill from the liquid handler used to determine true working volume, controlled freezing rates to mitigate the risk from ice plugs and, importantly, an awareness of sample tube features (capping options, burst pressure and working volumes).

Read, Share and Comment on this Article, visit: www.labmate-online.com