Maintaining sample integrity during repeated freeze/thaw cycles

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Storage of biological materials or newly synthesised compounds in -80°C freezers is now commonplace. The need to retrieve aliquots from these compound libraries or bio stores means that individual tubes must first be thawed and, following the aliquoting procedure, re-frozen. For popular, interesting, or useful samples this can result in multiple freeze/thaw cycles during the storage life of the sample. This paper sets out to discover if samples stored in quality screw-cap sample tubes rated for -80°C storage suffer any degradation from either very long-term storage or repeated freeze/thaw cycling.

To ascertain the facts about extended storage, representative sample storage tubes of four types were used and, in all cases, stored in -80°C freezer conditions. The testing had three main objectives:

- To confirm any changes to the sample storage volume that may occur throughout its lifecycle in a freezer
- To confirm no damage is likely to occur that could compromise the integrity of the tube during regular freeze/thaw cycles
- To re-affirm that the dual-threaded cap design successfully prevents leakage and evaporation over long periods of time

The tests were conducted on externally threaded tubes designed and manufactured for Azenta of 1.0mL and 1.9mL working volume, and 1.0mL & 3.8mL working volume treated with electron-beam (e-Beam) radiation.

Figure 1: Test samples stored in -80°C freezer.

The experimental design was as follows:

Apparatus and materials

Tubes selected for tests were Azenta Tri-Coded Tubes, External Thread, Pre-racked, Uncapped, or Capped (e-Beam treated only):

48 x 1.9mL Tri-coded Tube, 48-format, External Thread, e-Beam treated
48 x 3.8ml Tri-coded Tube, 48-format, External Thread
96 x 1.0mL Tri-coded Tube, 96-format, External Thread
96 x 1.0mL Tri-coded Tube, 96-format, External Thread, e-Beam treated
Either 1 x 48- or 1 x 96-format Azenta External Cap Carrier

1 x -80°C Freezer
1 x Torque-controlled capper
1 x 12-channel pipettor
1 x 500ml bottle of Ringer's Solution
1 x Precision laboratory balance

Method

Using the 12-channel pipettor, the maximum working volume (1,085µl) of Ringer's solution was aspirated into each of 96 tubes in the SBS rack. The capper was adjusted to apply 0.08 Nm of torque to each cap as it was transferred from the cap carrier to its tube, capping it accordingly.

Using the precision balance, each tube was weighed and the weight and rack position from A1 to H12 was recorded. A visual check of each tube was made for any possible damage, such as grazing or cracking of the polymer surface and the condition of the was noted in a log. The completed rack of 96 tubes was then transferred to the -80°C freezer.

The tubes were then withdrawn from storage at -80°C, at defined predetermined intervals as follows:

• 2 Weeks • 1 Month • 3 Months • 6 Months • 1 Year • 2 Years • 3 Years

At each sampling interval designated above, the SBS rack of tubes was removed from the freezer. The rack was then left out on the bench until the tubes had returned to room temperature. After stability at room temperature was achieved, each tube was re-weighed, and data was recorded.

At the same time, each tube was checked visually for any possible damage, grazing, or cracking, and anything noteworthy was recorded. After completion of all weighing and checks, the filled SBS rack of tubes was returned to the -80°C freezer.

The process was then repeated for each of the tube types at each incremental time point over three years, except the e-Beam sterile versions, which were only sampled after one year.

Results and observations

For the 1.0mL Azenta Tri-Coded Tubes, the total average weight loss of all 96 tubes was 0.010% (equal to 0.018mg) over the first two-week sampling period and only increased to a loss of 0.045% (equal to 0.088mg) over the full three-year test period.

Table 1: Results for 1.0mL in 96-position rack.

TIME OF WEIGHING	AVERAGE % CHANGE
Before Storage	100%
2 Weeks	-0.01%
1 Month	-0.01%
3 Months	-0.03%
6 Months	-0.03%
1 Year	-0.03%
2 Years	-0.04%
3 Years	-0.05%

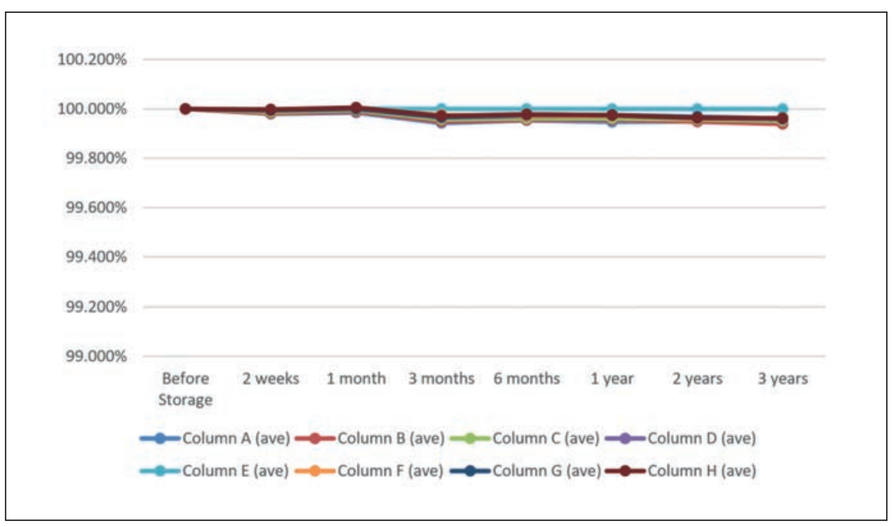

Figure 2: Variation between columns in the same 1.0mL tubes in a 96 rack over time.

There was no observed cracking, grazing, or other physical damage caused throughout the frozen storage period, nor by repeated freeze/thaw cycles over a three-year period.

This demonstrates the seal integrity of the 1.0mL Tri-Coded Tubes when correctly capped is adequate for long-term storage of aqueous-based substances, if the specified working volume is adhered to.

Using the above method, the test was repeated with the larger 1.9mL Tri-Coded Tubes in a 48-position SBS rack. The only experimental differences were that the volume aspirated – in this case 2,073µl of Ringer's solution to more closely match the correct working volume of this larger tube and the torque setting of 0.10 Nm for the larger tube and cap in use.

The total average weight change of all 48 x 1.9mL tubes was 0.006% (equal to 0.022mg) over two weeks, and then only increased to 0.06% (equal to 0.264mg) over three years.

Table 2: Results for 1.9mL 48-position rack.

Time of weighing	Average % change
Before Storage	100%
2 Weeks	-0.01%
1 Month	0.00%
3 Months	-0.04%
6 Months	-0.04%
1 Year	-0.04%
2 Years	-0.06%
3 Years	-0.07%

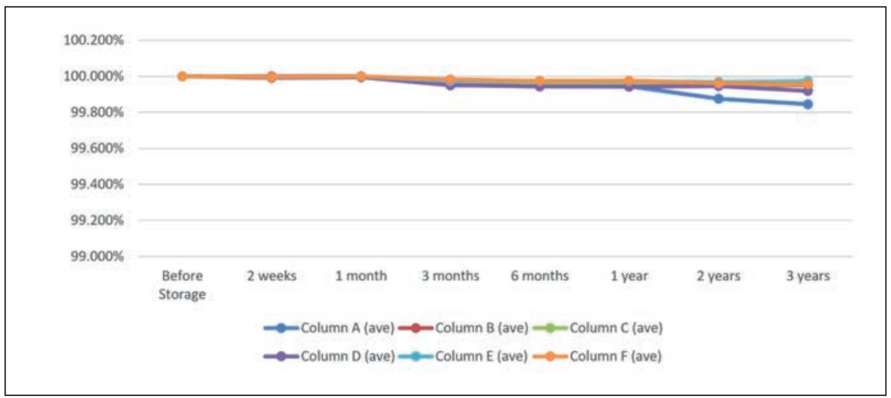

Figure 3: Variation between columns in the same 48 rack of 1.9mL tubes over time.

Again, there was no observed cracking, grazing, or other physical damage caused throughout the freezing period, nor by repeated freeze/thaw cycles over a three-year period.

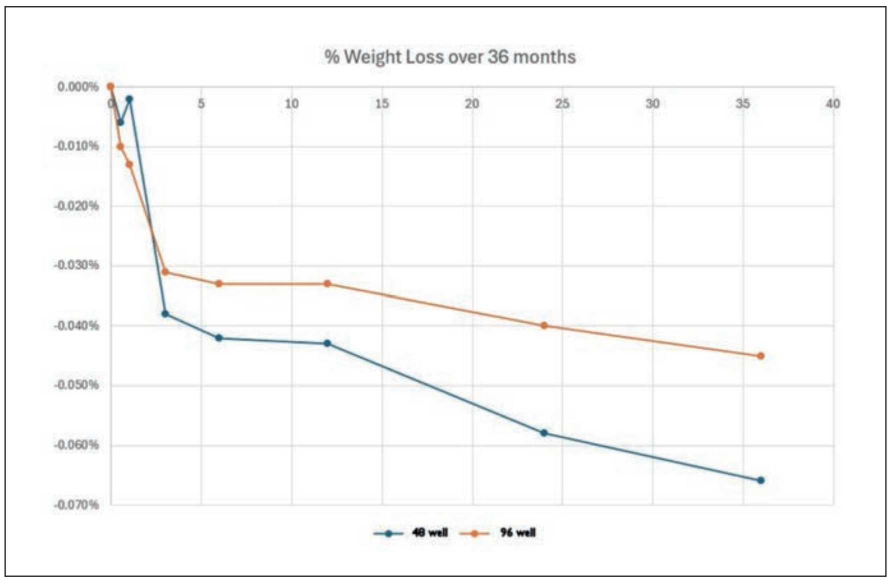

Figure 4: Comparing 1.9mL 48-position and 1.0mL 96-position Tri-Coded Tubes.

Effect of electron beam treatment

In the second part of the experiment, attention was turned to polymer tubes which had been treated using a standard dose of 62 kGy of e-Beam radiation. Both 1.0mL and 3.8mL treated tubes were tested, the key difference being that these treated tubes are supplied already capped. The experimental method for both was thus modified to include an initial step of decapping the racked tubes with the handheld single tube capper/decapper specified above. There were only two data points for these tests: initial weight and residual weight after one year.

The aspirated volumes in this case were:

1mL tube = 1.085mL
4mL tube = 4.150mL

The observed weight change after one year was as follows:

For the 96 x e-Beam treated 1.0mL Tri-Coded Tubes, pre-capped, pre-racked, the total average weight change of all 96 tubes was 0.006% (equal to 0.012mg) over one year.

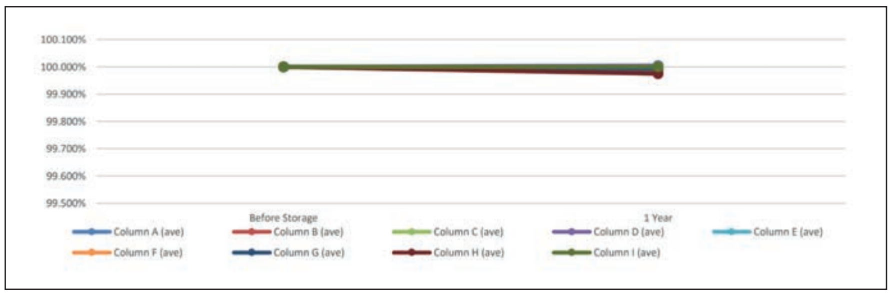

Figure 5: Variation between columns in the same sterile 48 -rack over one year.

For the 48 x e-Beam treated 3.8mL Tri-Coded Tubes, pre-capped, pre-racked the total average weight change of all 48 tubes was 0.0002% (equal to 0.0016mg) over one year.

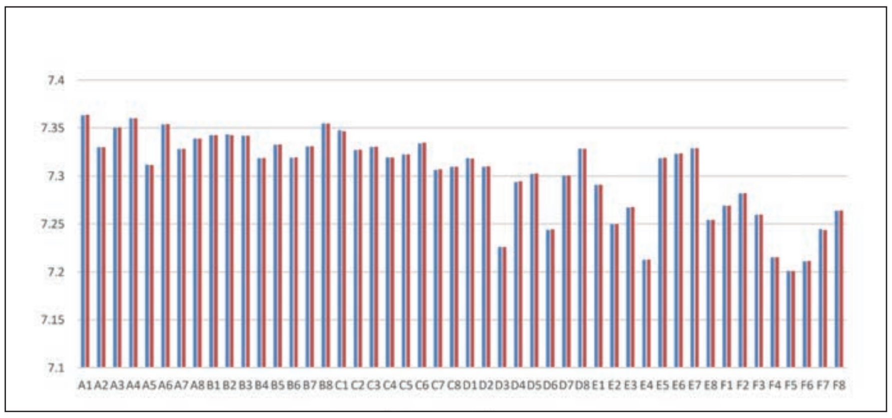

Figure 6: Comparison of weight change at individual tube positions in a 48-position rack.

Again, there was no observed cracking, grazing, or other physical damage caused throughout the freezing period, nor by repeated freeze/thaw cycles over a one-year period.

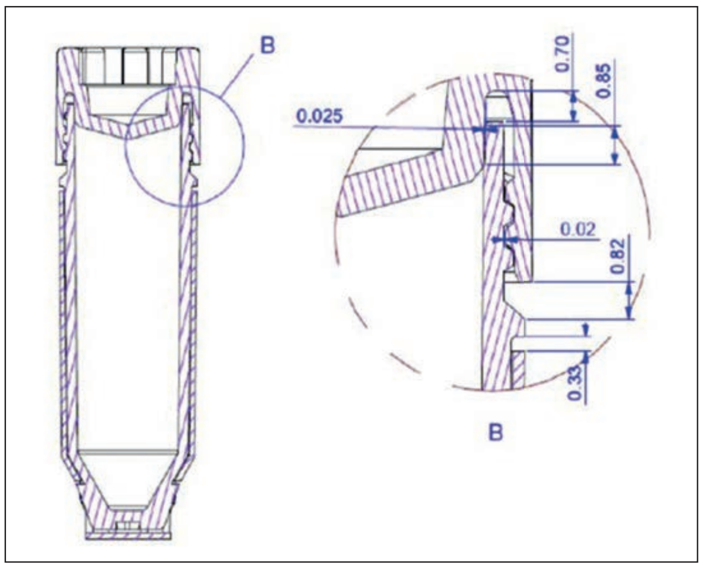

Figure 7: Schematic of the compression sealing design of an externally threaded tube with tolerances in millimetres.

Conclusions

After collection of three years of tube weight data for the 1.0mL and 1.9mL Azenta Tri-Coded Tubes, and annual data for the 3.8mL and 1.0mL Azenta e-Beam treated tubes, it suggests a similar trajectory and gives confidence to state that these products are suitable for long-term storage applications. Azenta Tri-Coded Tubes have both the cap and the tube body manufactured from the same polymer. This reduces potential leakage caused by the differential coefficients of expansion that can be seen when two different polymers are used. By standardising on the same polymer for construction throughout, any expansion or contraction during freeze/thaw cycles is the same for both parts of the article and the possibility of any gap opening between seal and tube is minimised.

It is important to state here that accurate capping to the specified torque values is necessary to avoid any irregular damage to the threads of both the tube and the screwcap, as well as guaranteeing a reproducible and leak-proof seal each time. The use of a double-start thread, which helps to prevent cross threading, and an ingenious compression seal design that also prevents over-tightening of the cap all contribute to the intrinsic integrity of the Azenta tube design and the very low losses seen over time as demonstrated above. The polymer used for these tubes is a polypropylene material with very low levels of extractables and leachables, to not contaminate the stored samples, especially those stored in solvent such as DMSO. Although free from DNA, DNA/RNA-ase, endotoxins, and pyrogens, the e-Beam process can also help to ensure sterility in the supplied product. Other treatment types such as gamma irradiation and ethylene oxide gas exposure can also be used to gain this benefit.

It is encouraging to also note that freeze/thaw cycles do not seem to cause any unnecessary damage to this type of quality sample storage tube if handled in a careful and appropriate manner, thus making them ideal for biobanking and compound management applications that require multiple access of samples over their lifetime.