



The clear advantage of using glass blood collection tubes for cell-free DNA preservation.

When deciding between blood collection tubes, one key factor for purchase can be the choice of glass or plastic. This decision can be difficult and is loaded with preconceived notions related to each material. One such issue is the too often overlooked considerable effect tube material has on preservative shelf life and blood draw volume.

Introduction

Blood collection tubes (BCTs) are a vital component of modern medicine. These devices are generally made of non-reactive glass, or more recently, polyethylene terephthalate (PET) (*GP34-A, 2010*) (*Bowen & Remaley, 2014*). There is a perception in the field that plastic is superior to glass when targeting cell-free DNA (cfDNA). This belief has not been formally addressed in the past and this paper is meant to better inform users of cfDNA stabilizing tubes.

Many reasons drive the switch from glass to plastic BCT. These include, but are not limited to: perceived increase in safety for laboratory workers (*GP39-A6, 2010*) and fear of OSHA citations and fines (*Ernst, 2003*). The manufacture of PET tubes is easier due to increased production speed related to injection-molded plastic. PET has recently become the material of choice for BCTs due to its low cost, clarity, and un-breakability (*Bowen & Remaley, 2014*).

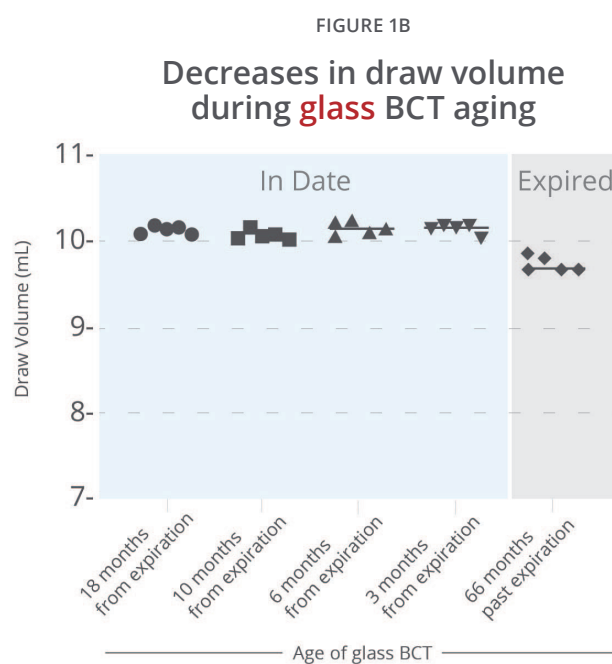
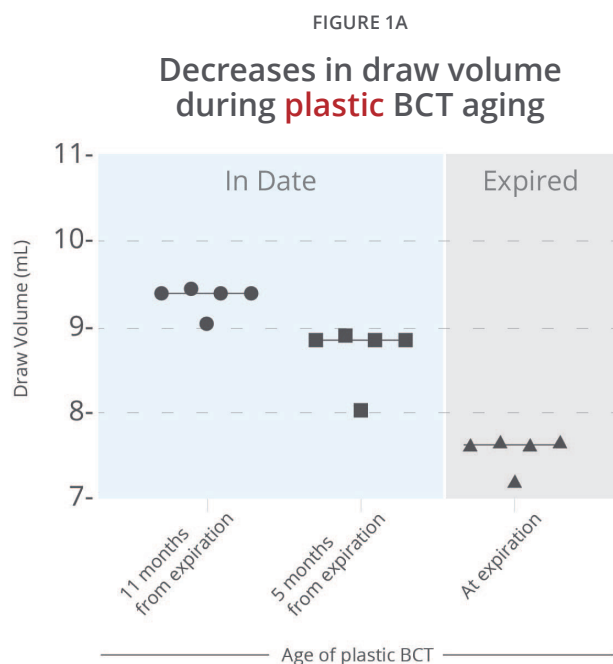
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Key Plastic Limitations

A key limitation with using a PET plastic tube is that it has poor vacuum retention and high water permeability, leading to liquid reagents evaporating and drying out in the tube over time (Bowen & Remaley, 2014).

To illustrate this point, we collected water into five tubes for each of three lots of common plastic blood collection tubes of various ages. There was a substantial change in the draw volume as the tubes aged, even while still in date (Figure 1a). This decrease in draw volume will affect the amount of recoverable plasma, thus affecting the sensitivity of certain cfDNA-based assays.

In contrast, the drawn amount of water in glass tubes was maintained and vacuum was retained throughout dating and for years after expiration. (Figure 1b).



Most stabilizing reagents are a liquid at the time of manufacturing. However, when a liquid reagent is placed into a plastic tube it loses moisture and turns into a viscous gel as the product ages. Because of the water retention properties of glass, Streck's Cell-Free DNA BCT® reagent remains a liquid even years past expiration (Figure 2).

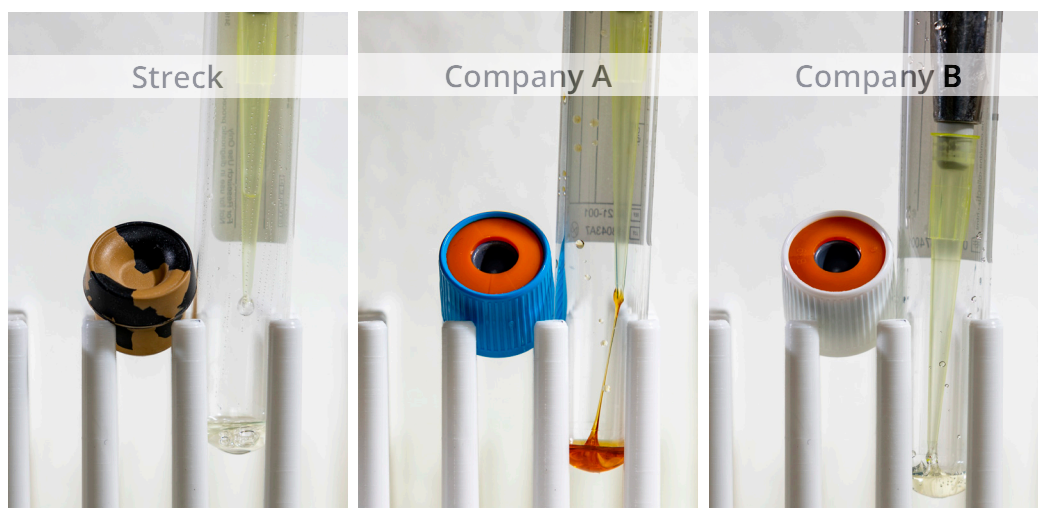


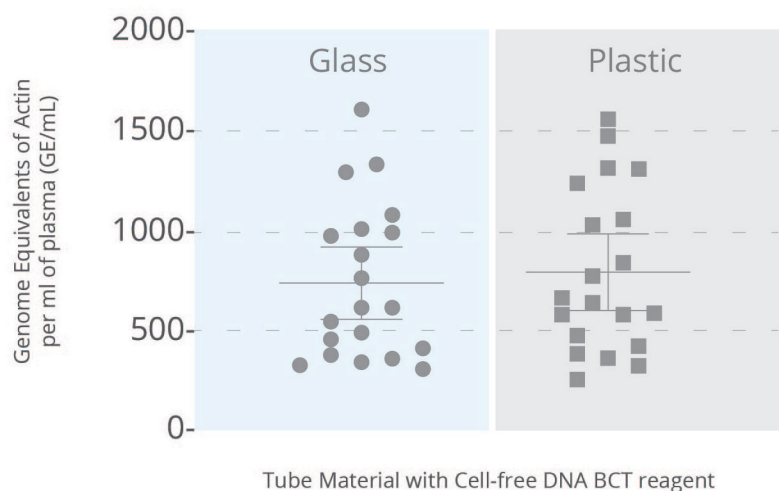
FIGURE 2

Cell-free DNA Yield

With an understanding that DNA binds to glass, a reduction of cfDNA quantities isolated from plasma has been assumed. Multiple studies have been conducted to determine if glass or plastic alters or interferes with clinical assays (Flanders, Crist, & Rodgers, 2003) (Preissner, et al., 2004) (Kratz, Stanganelli, & Van Cott, 2006) (Bowen & Remaley, 2014) (Bowen, Sattayapiwat, Gounden, & Remaley, 2014). None of the studies found a clinically significant difference when comparing glass vs. plastic usage for clinical assays. None of the studies, however, looked at cfDNA. We isolated the cfDNA from equal amounts of plasma from 20 self-declared healthy donors and used a Droplet Digital™ PCR assay targeting actin to examine differences in the amount of cfDNA. In a study using the same Cell-Free DNA BCT stabilization reagent spiked into either glass or plastic BCTs, no differences were observed (Figure 3), illustrating plastic does not offer any advantage over glass in terms of cfDNA quantity.

FIGURE 3

ddPCR of Actin



Conclusion

As demonstrated, poor vacuum retention and loss of moisture shortens the shelf life of PET stabilization tubes and can lead to inconsistent results as the product ages. In contrast, glass BCTs maintain vacuum and have better moisture retention, leading to a long shelf life. The brief study above shows plastic does not offer any advantages when isolating cfDNA. It must be stated that PET, functionally, has a host of shortcomings that are all too often overlooked. In summation, a glass BCT — designed specifically for cfDNA — provides the best shelf life, performance near expiration, sample draw volume, and similar cfDNA yields when stabilization is required.

References

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