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Sample Integrity Compendium: How Safe are Your Samples?

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How Safe are Your Samples? Part I: Evaluation of FluidX External Thread Tubes for Potential Leachable Compounds

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INTRODUCTION

Plastic storage tubes are used in laboratories worldwide to store biological and chemical samples. As most plastics are supplied sterile, pyrogen- and DNAse/RNAse-free, it is generally accepted that this guarantees product integrity. Few researchers ever consider that despite these apparent guarantees, plastic ware can still provide a potential source of error.

Evidence shows that bioactive compounds can diffuse into solutions that come into contact with the surface of the plastic [1,2]. These compounds, typically referred to as “leachables” or “extractables”, are used during the manufacturing process to improve product stability and durability. However, these compounds can have a significant impact on scientific experiments and pose a likely source of error in many assay systems. Examples of leachable interference include inhibition of enzyme activity and falsification of nucleic acid quantification.

The aim of this evaluation was to determine if “extractables” could be detected in solvent solutions stored in FluidX external thread tubes and to compare the performance against competitor tubes.

DEFINITIONS

“Leachables” - Compounds that leach from a closed container into a sample as a result of direct contact.

“Extractables” - Compounds that can be extracted from a closed container when in the presence of a solvent.

MATERIALS

The following storage tubes were used in the evaluation:

- 0.7ml external thread transparent tube – FluidX (n=3)
- 0.7ml internal thread transparent tube – Manufacturer T (n=3)
- 0.7ml internal thread transparent tube – Manufacturer M (n=3)
- 0.7ml & 1.3ml internal thread transparent tube – Manufacturer L (n=2 + n=2)

METHODS

1. Tubes were filled with 80% ethanol and the caps screwed on
2. Tubes were then placed into centrifuge tubes
3. Using a shaker table, the tubes were shaken at 20°C at 120rpm for 24 hours
4. 1µl was eluted from each tube
5. Elutions were subjected to Gas Chromatography / Mass Spectrometry analysis (Thermo Fisher Co. Ltd. GC:TRACF1300 / MS:ISQ).

All testing was performed by an independent 3rd party laboratory: Ig-M Co., Ltd. Headquarters Lab, Hyogo Prefectural Institute of Technology, Japan.

RESULTS

FluidX - 0.7ml External Thread Tubes (n=3)

GC Analysis: Peak (mins)	Elution 1	Elution 2	Elution 3	MS Analysis: Substance Identified
No peaks detected	✓	✓	✓	No dissolved substances identified

Manufacturer T - 0.7ml Internal Thread Tubes (n=3)

GC Analysis: Peak (mins)	Elution 1	Elution 2	Elution 3	MS Analysis: Substance Identified
10.28 - 10.29	✓	✓	✓	Benzoic acid, 4-ethoxy-, ethyl ester ($C_{11}H_{14}O_3$)

Manufacturer M - 0.7ml Internal Thread Tubes (n=3)

GC Analysis: Peak (mins)	Elution 1	Elution 2	Elution 3	MS Analysis: Substance Identified
4.20 - 4.26	✓		✓	Pentadecane ($C_{15}H_{32}$)
8.47 - 8.49	✓	✓	✓	Benzaldehyde, 3,4-dimethyl- ($C_9H_{10}O$)
9.11	✓	✓	✓	1-Dodecanol ($C_{12}H_{26}O$)

Manufacturer L - 0.7ml Internal Thread Tubes (n=2) + 1.3ml Internal Thread Tubes (n=2)

GC Analysis: Peak (mins)	Elution 1	Elution 2	Elution 3	Elution 4	MS Analysis: Substance Identified
9.05 - 9.11	✓	✓	✓	✓	1-Dodecanol ($C_{12}H_{26}O$)

DISCUSSION

The evaluation showed that ethanol was an effective solvent for detecting leachables in the sample storage tubes.
From all the eluted material samples, at least 2 elutions of the same tube type have common properties. It is, therefore, possible to ensure the authenticity of the data.
From MS analysis of the elution, the most likely substance was presented for each manufacturer. More certainty can be determined by using the appropriate reagent as a standard substance for comparison and comparing the measurement.

CONCLUSION

The evaluation demonstrated that there were obvious differences in performance of sample storage tubes between manufacturers. Since leachable compounds from plastic resin can have an adverse and erroneous effect on scientific investigations, it is vital that the performance characteristics of the storage tube are carefully considered.
FluidX external thread tubes were the only tubes that consistently produced no detectable leachables and, therefore, offer a superior solution for long-term storage of biological and chemical samples.

REFERENCES

1. A. Olivieri, O.S. Degenhardt, G.R.McDonald, D. Narang, I.M. Paulsen, J.L. Kozuska and A. Holt. Can. J.Physiol. Pharmacol. 2012; 90: 697-703.
2. McDonald GR, Hudson AL, Dunn SM, You H, Baker GB, Whittal RM, Martin JW, Jhe A, Edmondson DE, Holt A. Science 2008; 322(5903):917.

How Safe are Your Samples? Part II - Working Volume

How Safe are Your Samples? Part II - Working Volume

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INTRODUCTION

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

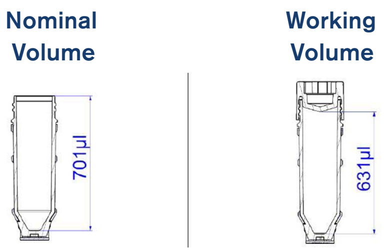
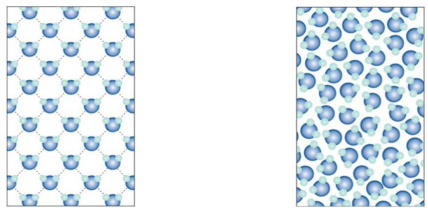


Figure 1

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.



Water molecules in solid ice *Water molecules in liquid water*

Figure 2

A consequence of this is that the volume occupied by water increases by approx 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 the graph below, 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).

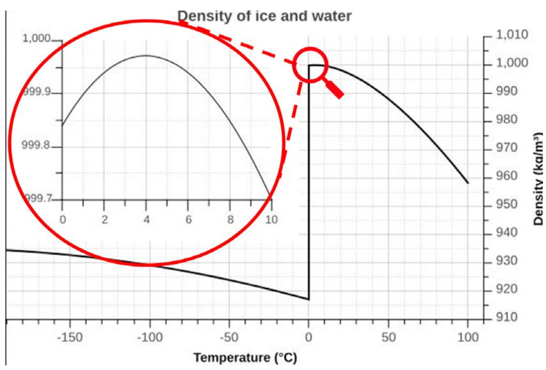


Figure 3

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

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

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)

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.

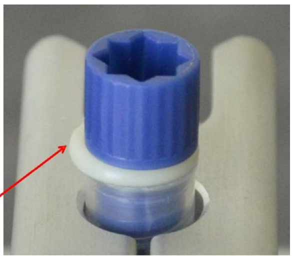


Figure 4

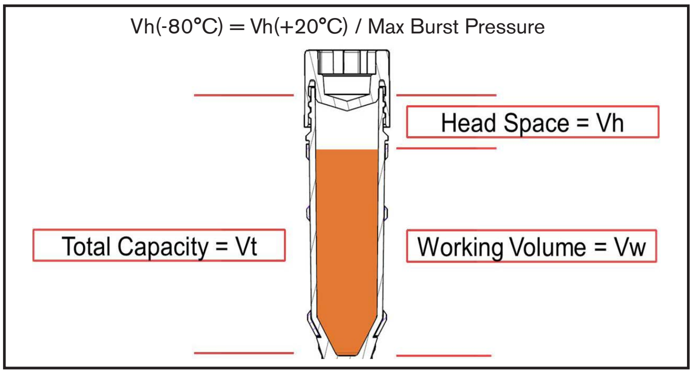
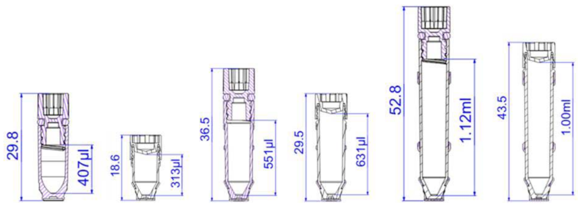


Figure 5

To address this issue, FluidX exploited the co-molding 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.

FluidX 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.

As can be seen by the table in figure 6, External Thread tubes offer significant working volume advantages over Internal Thread tubes.



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 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.

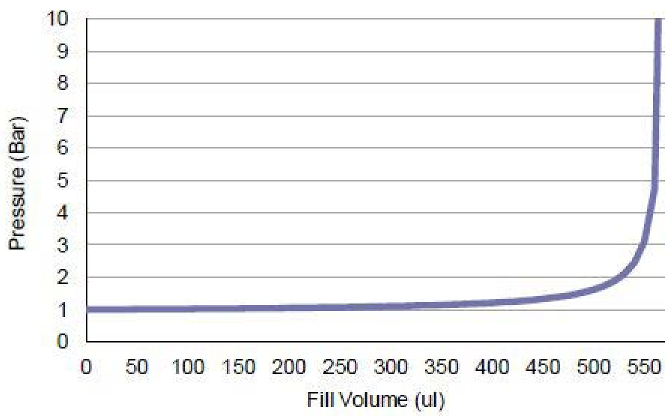


Figure 7

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.

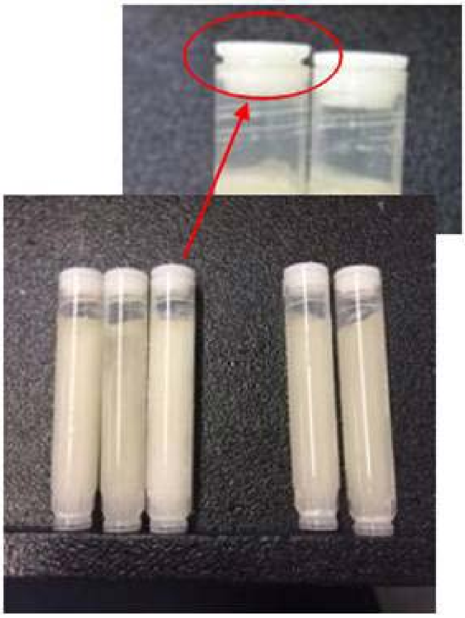
Figure 8

The maximum fill volume should to be 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.

Sample at 20°C	Sample at Freezing Temp
• Sample Volume = 530ul	• Sample Volume = 578ul
• Headspace Volume = 101ul	• Headspace Volume = 53ul
• Air Volume = 101ul	• Air Volume = 95ul
• Internal Pressure = 1 Bar	• Internal Pressure = 2Bar
	Sample Expands by approximately 9%
	Air in Head Space Shrinks by approximately 5%

Figure 9



Example of TPE cap popping during thawing

Figure 10

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.

Figure 11

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.

Brooks offers a range of products that enable controlled rate freezing through the Biocision range. Products are identified in the following posters: "Best Practices for introducing -80°C Bio-Samples into -190°C Vapour Phase LN2 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

Sample Freezing in controlled conditions	Sample Freezing in uncontrolled conditions
Sample should be frozen from the bottom to allow the liquid to expand up the tube. If the sample is frozen from the top a plug will form preventing the sample from expanding upwards forcing the sample to expand outwards which can lead to tube wall damage.	Care should be taken when freezing samples in a forced air cooling chamber that the sample does not freeze from the top. This causes a frozen plug to be formed at the top driving the liquid expansion outwards, potentially causing damage to the tube.

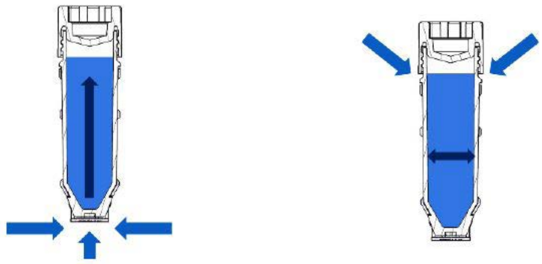


Figure 13

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).

How Safe are Your Samples? Part III: Sample Integrity Through Secure Capping

How Safe are Your Samples? Part III: Sample Integrity Through Secure Capping

Figure 1

INTRODUCTION

When using sample tubes, secure capping is paramount to protect sample integrity during the storage and handling process for a range of applications. These applications could include; Biobanking, Compound Management, Cell Therapy, Benchtop Research or applied industry. Two major hazards that can be mitigated through safe and secure sample tube capping are:

- Evaporation
- Cross contamination

EVAPORATION AND CROSS CONTAMINATION

Evaporation and cross contamination can occur either directly through the tube and cap or through a mechanical failure or flaw. During long-term storage or handling, aqueous samples can evaporate or cross contaminate each other potentially causing irreplaceable samples to be lost or compromised, rendering them unusable for future research or treatment. In closed customer testing FluidX Sample Tubes have shown to provide higher sample integrity through lower levels of evaporation.

To ensure sample integrity, FluidX Sample Tubes have been leak tested and have been through rigorous testing methods both under vacuum (to test the mechanical seal of the tube and cap) and under pressure (to simulate an increase of internal tube pressure).

Under Pressure:

- Product samples are taken from each cavity of the tool, at the start, end, and during batch production
- The tube is connected to a silicone pressure tubing manifold via a hole drilled in the tube base
- The cap is applied with the minimum recommended torque for the tube and cap
- The manifold is then attached to a test rig and a pressure of 2Bar is applied
- The pressure must be maintained for 10 seconds without any reduction in pressure observed.

Under Vacuum:

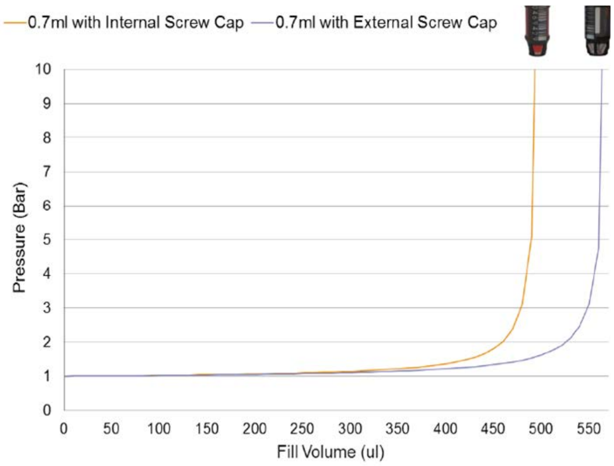
- Product samples are taken from each cavity of the tool, at the start, end, and during batch production
- Tubes are filled with 0.15ml of with blue pigment. (Ethanol is used due to its low surface tension which will accelerate and exaggerate any leaks)
- The tubes are then capped, just below the minimum recommended torque level, and independently weighed
- The tubes are then placed on their sides in a tray lined with filter paper and placed in a vacuum chamber. A vacuum of -0.03Mpa (-19psi) is applied
- Tubes are left for 24 hours
- The sample is re-weighed and the weight compared with the original recorded
- Tubes and caps pass QC when there is no evidence of sample loss.

Quality control processes ensure that every batch of FluidX Sample Tubes are above industry standard to provide safe and secure long-term storage for precious samples. If any of the tubes tested fail then the entire batch fails quality control and is inspected to mitigate any issues in future.

The rationale for these two tests is to maintain sample integrity during periods of increased internal pressure and to assure reliability of seal from the cap.

INTERNAL THREAD VS. EXTERNAL THREAD

As aqueous samples expand when frozen, internal pressure is increased and burst pressure is paramount to calculate the safe working volume of each tube (see: How Safe Are Your Samples? Part II: Working Volume). When freezing samples, it is imperative to know the maximum fill volume of the tube being used as, when water based samples are frozen, they expand by approximately 9%. This is an important factor when selecting an internal or external thread tube & cap, Figure 2 shows the results of comparative testing with 0.7ml FluidX sample tubes.



Graph showing the pressure generated with increasing fill volumes
In both an internal and external thread 0.7ml jacket sample storage tube

Figure 2

The results show that due to the higher headspace volume in the FluidX External Thread Sample Tube, the internal pressure is lower and therefore a greater volume of sample can be stored with a lower risk of sample loss through bursting when sealed with an external cap. Furthermore, results show that the FluidX External Thread Sample Tube offers more protection from evaporation.

Figure 3 Shows the results of testing on average change in weight over one month with three capping options; traditional Internal O-Ring Cap , New FluidX External Cap and the New FluidX Internal Co-Mold Cap. Both the New External Cap and New Internal cap demonstrate excellent sealing, whilst the traditional Internal O-Ring Cap showed significant evaporation after one month.

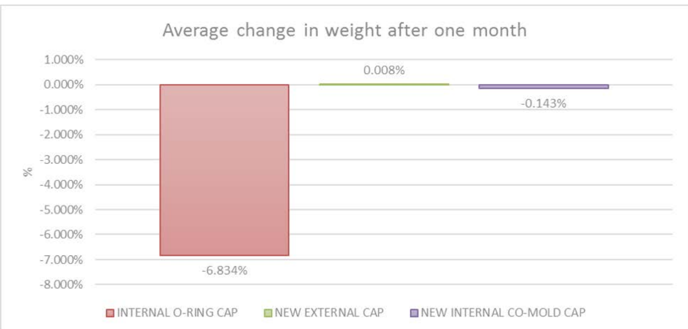
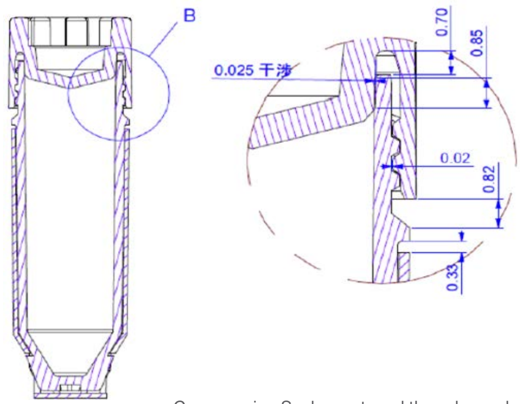


Figure 3

The FluidX External Thread Sample Tube was designed for a greatly increased burst pressure for enhanced sample integrity, it also has a range of other benefits including:

- Increased working volume allowing users to store more sample
- Reduced tube height enabling a higher density of storage
- Prevention of cross-threading and 'jumping' if the tube is over-torqued
- Improved robustness of automated capping and decapping processes

These improvements are only possible with highly advanced manufacturing techniques, and therefore a rigorous quality control process. Details of the tolerances can be seen in figure 4 below.



Compression Seal on external thread sample storage tube

Figure 4

SUMMARY

Due to the advanced manufacturing techniques used when FluidX tubes and caps are manufactured the results demonstrate the superiority of the New Internal Co-Mold Cap and New External Cap over the traditional Internal O-Ring Cap. This shows how two of the major hazards to compromising sample integrity; evaporation and cross contamination can be mitigated with the use of an effective capping method.

Reliable and robust capping can be assured through high quality manufacturing processes including a rigorous quality control process, simulating the expansion of aqueous samples during freezing and testing mechanical leaks.

The results all indicate that the new FluidX External Thread Cap provides an assured level of quality to maintain sample integrity whilst offering a range of other benefits including; higher density of storage, enabling automation, manual handling and increased working volume vs. internal thread tubes of the same size.

Brooks Life Sciences, a division of Brooks Automation (Nasdaq: BRKS), is the leading worldwide provider of innovative and comprehensive sample lifecycle management solutions for the bioscience industry.

These solutions include our automated storage systems, sample consumables and instruments, cell cryopreservation storage products, onsite and offsite temperature-controlled storage models, global sample preparation and analytical lab services, and innovative informatics systems and technology services.

Our team of sample management experts delivers high quality, flexible sample management products, services and technology solutions that support hundreds of bioscience customers around the world, including the top 20 biopharmaceutical companies. Our industry-leading sample management solutions enable our customers to unlock sample intelligence and advance scientific research in order to deliver healthier and brighter tomorrows to individuals around the world .

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