N° 21

FORUM

TECHNICAL NOTES & APPLICATIONS FOR LABORATORY WORK

CONTENT

SAMPLE STORAGE TUBES AS QUALITY-CRITICAL COMPONENTS IN BIOBANKING

1/ INTRODUCTION

Biobanks store biological samples and associated data to make them available for clinical studies as well as research on biomarkers, personalised medicine and public health.

The paramount goal of biobanking is the preservation of sample integrity during all steps of sample collection, processing, storage and delivery; hence, factors influencing the sample quality during these steps need to be understood and controlled.

Whereas storage temperatures and environments as well as methods for secure sample tracking were always considered important factors in biobanking, the actual sample containing tubes have long been disregarded. Here we emphasise the importance of sample tubes as critical components of biobanking. In particular, Cryo.s Biobanking Tubes are analysed with regards to cleanliness of tube raw material, tube closure, biomolecule adsorption onto the tube surface and quality of the datamatrix barcodes used for tube identification and tracking.

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2/ POLYMER QUALITY AND LEACHABLES

2.1/ BACKGROUND

Multiple additives and agents can be utilised to alter the physical properties and processability of polymers used for labware production. Such chemicals are:

- **UV stabilisers (e.g. benzophenone, benzotriazole, oxalanilide),**
- **antioxidants (e.g. organo phosphites),**
- **thermo stabilisers,**
- **nucleation starters,**
- **plasticisers (e.g. phthalate esters),**
- **mold release agents,**
- **antistatic agents,**
- **irradiation protectors,**
- **clarifiers.**

Several studies have shown that polymer additives or their degradation products can migrate out of polymer-based microtiter plates or tubes and affect the outcome of biochemical assays performed with these products[i ii iii] .

There is the potential risk of similar phenomena occurring with biological samples stored in polymer tubes over long periods of time. Thus, storage tubes used in biobanking should be made of high-quality, virgin polymers with the least possible contents of additives and leachable substances.

Virtually all polymer-based cryogenic storage tubes are made of polypropylene – a polymer proven to be excellently suited for the application at ultra-low temperatures.

Today, an immense variety of different polypropylene qualities are available, although these raw materials widely differ in chemical composition and physical properties, as well as purity and certification. Greiner Bio-One uses a medical grade, USP class VI certified polypropylene type for the production of all Cryo.s Biobanking Tubes.

In this context, the attribute 'medical grade' refers to the comprehensive certification of the material (e.g. European Pharmacopeia 3.1.3, 3.1.6 and 3.2.2), special cleaning processes before production start and more than 15 years of history of the raw material with unchanged polymer composition. The USP class VI certification refers to a biocompatibility testing in accordance with the United States Pharmacopeia (USP). Primarily, passing this test is indicative of a high biocompatibility of the tested raw material; secondarily, it suggests a low content of leachables in the material. In addition, the raw material of Cryo.s Biobanking Tubes is certified free of the following chemical elements and agents:[1]

- **heavy metals,**
- **phthalate esters,**
- **mold release agents,**
- **antistatic agents,**
- **TSE and BSE.**

Manufacturer Code	Tube Code	Extraction and IR spectrum to check for amide	Extraction and complete GC/MS scan
Greiner Bio-One	G1 (300 µl Cryo.s Biobanking Tubes, non-sterile)	•	•
A	A1	•	-
B	B1	•	-
C	C1	•	-
D	D1	•	-
	D2	•	-

Table 1: 96-way cryogenic storage tubes from different suppliers were tested for leachables.
This table provides an overview on leachable tests performed with selected tubes from Greiner Bio-One and other suppliers. Except for Greiner Bio-One all other tube manufacturers are anonymised and tube codes are utilised to differentiate between different tube types offered by individual manufacturers (e.g. manufacturer 'D' offers the two different tube types 'D1' and 'D2').

2.2/ METHOD

In order to assess the potential contaminations of storage tubes with amides (often used as mold release agents in injection molding), six different cryogenic storage tubes **(see table 1)** were extracted with 50 % ethanol in water for nine days at room temperature. Subsequently, the extractable material was characterised by IR spectroscopy after complete evaporation of the solvent. Typically amides yield signals in the infrared wavenumber bands between 1630 and 1690 cm^{-1} (C=O stretch) and between 3300 and 3700 cm^{-1} (N-H stretch). In a second leachable test, Greiner Bio-One's Cryo.s Biobanking Tubes (300 µl version, non-sterile version) were extracted with four different solvents: 10 % ethanol/water, acetic acid-acetate-buffer (pH 4.6), Tris-EDTA-buffer (pH 8.0) and 100 % DMSO. This extraction was carried out over 72 h at 37 °C. Subsequently complete GC/MS fingerprints were recorded. This analysis was performed by UL International GmbH (Ochsenhausen, Germany).

2.3/ RESULTS

The IR spectra of ethanol extracts indicated the potential contamination of tube type 'B1' from manufacturer 'B' with amides. A mild contamination with amides was also detectable for the tubes 'D1' and 'D2' from manufacturer 'D'. All other tube extracts did not reveal amide-specific peaks in their IR spectra **(figure 1)**. The full GC/MS footprint of four different extracts (1. ethanol/water, 2. Acetic acid-acetate-buffer pH 4.6, 3. Tris-EDTA-buffer pH 8.0, 4. 100 % DMSO) of Greiner Bio-One's Cryo.s Biobanking Tubes revealed no extracted substances in any of the four extracts **(figure 2)** to undermine the high purity of the raw materials used for producing these tubes.

1 All 'free of' statements are based on supplier information and formulated to the best of Greiner Bio-One's knowledge and understanding.
Note: Detection limits apply for each 'free off' statement. Contaminants may be present in concentrations below detection limits.
Some statements depend on detection methods with individual detection limits. For further details and actual certificates please contact your Greiner Bio-One representative.

2.4/ CONCLUSION

Greiner Bio-One utilises a high-quality, virgin and pure polypropylene type for the production of Cryo.s Biobanking Tubes. Extraction of these tubes with four different solvents revealed no detectable substances in the corresponding GC/MS fingerprints. Exemplified based on the presence of amide-specific IR signals in ethanolic extracts from some tube types, it becomes evident that the absence of leachable additives is not a guaranteed feature of tubes intended for sample storage in biobanking. Rather, raw material types vary between individual tube manufacturers and commonly accepted standards, such as the use of materials with low additive content, are still missing.

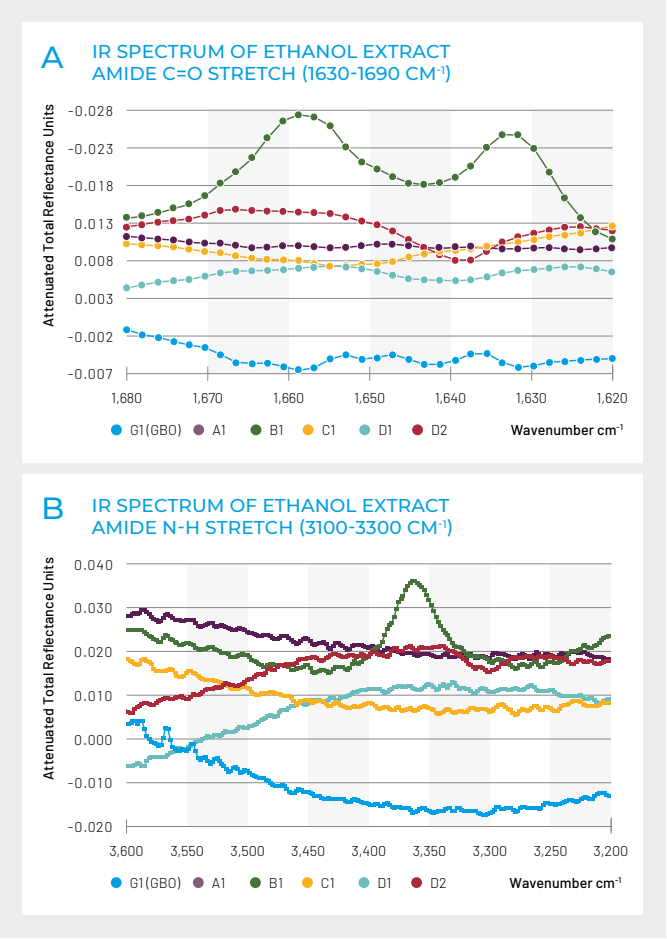

Figure 1: Extraction of cryogenic tubes with 50 % ethanol in water with subsequent IR-spectroscopical analysis revealed amide-specific signals in the extract from tube 'B1' and mild signals in the extracts from tubes 'D1' and 'D2'.
(A): IR spectrum from 1620-1690 cm^{-1}.
(B): IR spectrum from 3220-3600 cm^{-1}.

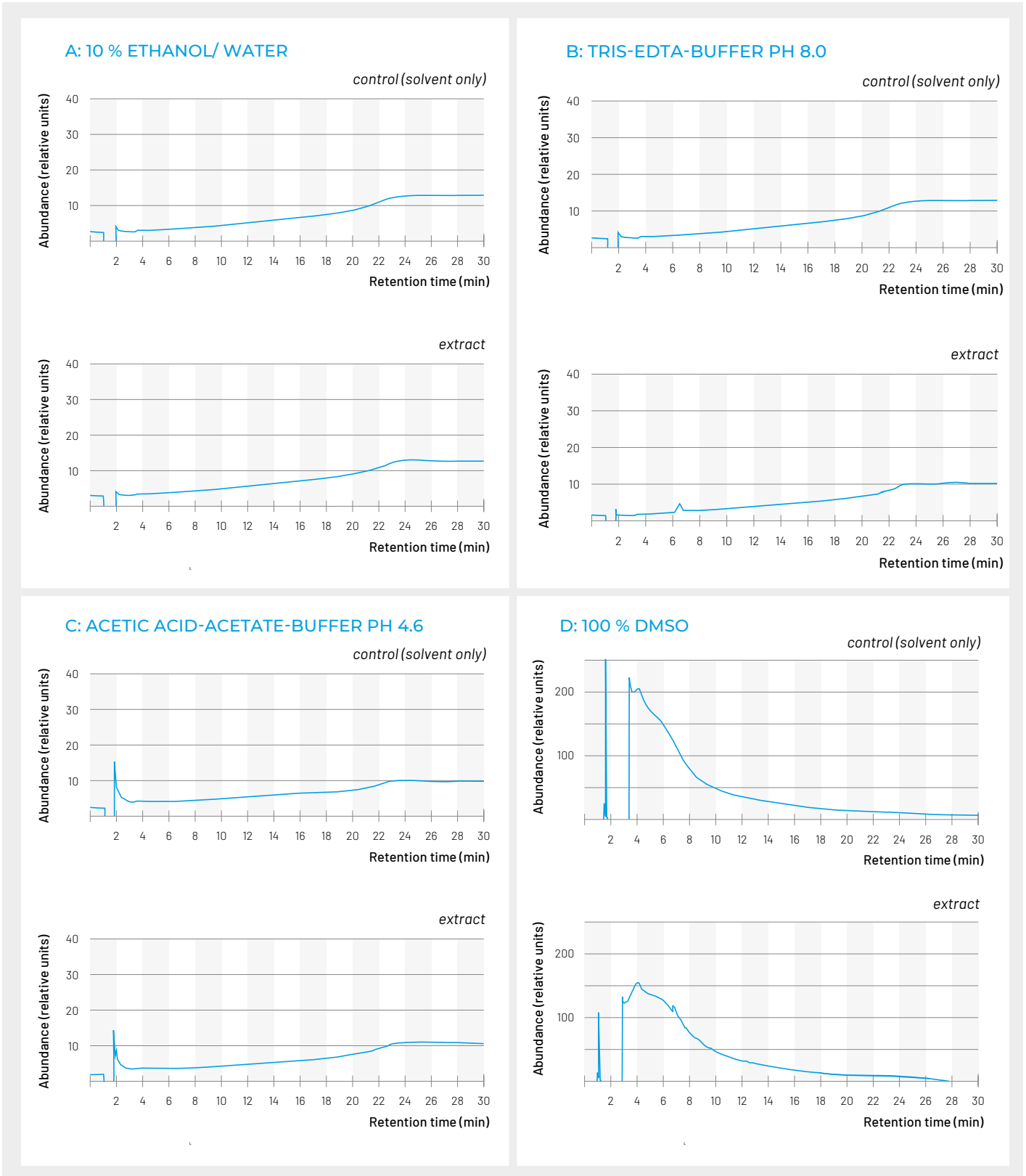

Figure 2: Extraction of Cryo.s Biobanking Tubes with four different solvents and subsequent GC/MS footprint analysis of the extracts.
Extraction with 10 % ethanol/water **(A)**, basic buffer solution (Tris-EDTA-buffer pH 8.0, **(B)**), acidic buffer solution (Acetic acid-acetate-buffer pH 4.6 **(C)**) and 100 % DMSO **(D)** revealed no significant extractable substances.

3/ BIOMOLECULE ADSORPTION ONTO STORAGE TUBE SURFACES

3.1/ BACKGROUND

In biobanking, the concentration of biomarkers in liquid samples (e.g. blood serum) should remain unchanged over the entire period of sample processing and storage. However, there is a general risk of certain biomarkers binding onto the surfaces of tubes used for sample aliquotation and storage. Such unintended sample adsorption onto tube surfaces depends on the actual type of biomolecule (e.g. nucleic acid, polysaccharide, lipid, peptide, protein etc.), as well as the type of tube raw material[iv] and details of the tube manufacturing process. In order to assess the adsorption of biomolecules onto storage tube surfaces, we have chosen a Iodine^{125}-labelled derivative of the peptide Insulin-like growth factor 1 (IGF1^{I125}) as the test substance and developed a radioactivity-based adsorption assay **(figure 3)**.

3.2/ METHOD

Humane IGF 1 was labelled with I^{125} on tyrosine residues applying the lactoperoxidase method, purified by HPLC (Dr. Carsten Tober, rent-a-lab, Reutlingen/Germany) and dissolved in fetal calf serum (FCS) to yield an IGF 1^{I125} concentration of 33.3 ng/ml. Disc-shaped material samples (Ø = 5.2 mm) were punched out of the side walls, **(figure 3, A)** of cryogenic storage tubes from different suppliers **(table 2).**

Per supplier three tubes were analysed. The discs were placed into the wells of 6-well plates. The internal side of each material sample (originally facing towards the tube's interior) was incubated with 30 µl test solution overnight in at -80 °C **(figure 3, B)**. After incubation, test solution was completely removed and the material samples were put into the wells of a 96-well plate with scintillation fluid. Emitted gamma-radiation was quantified and adsorbed IGF1^{I125} determined based on standard curves derived from known amounts of IGF1^{I125} **(figure 3, C).**

3.3/ RESULTS

The two tested sterile tube versions 'G2' and 'D1' revealed higher IGF 1I125 adsorption than the tested non-sterile tube types 'A1', 'G1', 'B1' and 'C1' **(figure 4)**. Whereas 'G2' and 'D1' adsorbed about 12 % of the IGF1^{I125} present in the serum sample used for incubation, the non-sterile tube types 'A1', 'G1', 'B1' and 'C1' adsorbed only 3.5 – 5 % of the contained IGF 1^{I125}. One non-sterile tube type, namely tube 'D2', revealed a >4 times higher adsorption than the average of all other tested non-sterile tubes. With this tube an absorption rate of 17.5 % of the present IGF 1^{I125} was observed.

Manufacturer Code	Tube Code	Extraction and IR spectrum to check for amide	Extraction and complete GC/MS scan
Greiner Bio-One	G1 (300 µl Cryo.s Biobanking Tubes, non-sterile)	•	•
	G2 (300 µl Cryo.s Biobanking Tubes, sterile)	•	•
A	A1	•	-
B	B1	•	-
C	C1	•	-
D	D1	•	-
	D2	•	-

Table 2: **96-way cryogenic storage tubes from different suppliers were tested in the IGF 1^{I125} adsorption test.**
Except for Greiner Bio-One all other tube manufacturers are anonymised and tube codes are utilised to differentiate between different tube types offered by individual manufacturers (e.g. manufacturer 'D' offers the two different tube types 'D1' and 'D2').

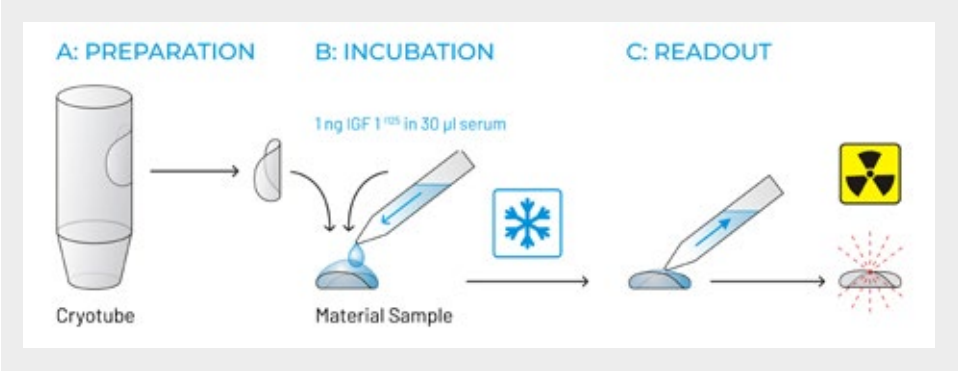

Figure 3: **Experimental procedure of the adsorption assays** with material sample preparation **(A)**, material sample incubation with radioactively labelled test substance (IGF 1^{1125} in serum, -80 °C, **(B)**) and quantification of emitted radiation from adsorbed IGF 1^{1125} **(C)**.

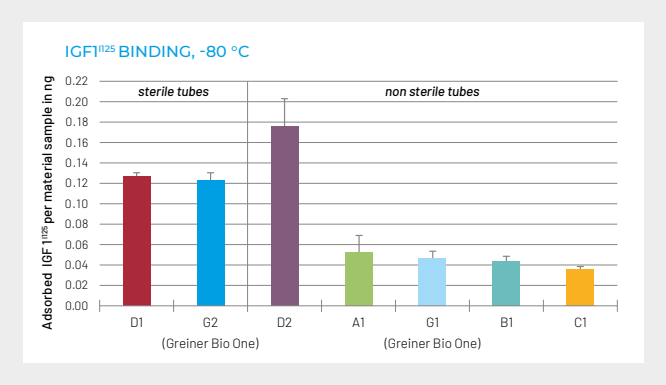

Figure 4: **Amount of IGF 1^{1125} adsorbed onto material samples** (5.2 mm-discs) after incubation at -80 °C with 30 µl FCS containing a total of 1 ng IGF 1^{1125}. Error bars indicate standard errors of the mean (n=3).

3.4/ CONCLUSION

Irradiation-based procedures regularly used for the sterilisation of cryogenic storage tubes bear the potential risk of introducing unwanted binding sites for biomolecules which then may be adsorbed onto the product surface.

The IGF 1^{1125} adsorption assay presented here indeed indicates higher IGF 1^{1125} binding onto sterile tube versions (Greiner Bio-One and manufacturer 'D') as compared to the average non-sterile tube. Although non-sterile tubes may be regarded as a gold standard for liquid sample storage, one non-sterile tube (tube 'D2') from manufacturer 'D' yielded above-average IGF 1^{1125} adsorption at -80 °C.
The data shown here indicate differences in IGF 1^{1125} adsorption onto tube surfaces depending on the tube type and sterilisation. It is likely that other classes of biomolecules reveal adsorption characteristics other than those of IGF 1^{1125} – a subject remaining open for future analyses.

Manufacturer Code	Tube Code	Type of tube closure (according to figure 5)
Greiner Bio-One	G1 (300 µl Cryo.s Biobanking Tubes, non-sterile)	Internal, silicone
	G3 (1000 µl Cryo.s Biobanking Tubes, non-sterile)	Internal, silicone
A	A1	Internal, TPE
	A2	External, sealing lip, no separate sealing material
B	B1	External, sealing lip, no separate sealing material
	B2	Internal, silicone
C	C1	Internal, silicone
	C2	Internal, silicone
D	D1	Internal, TPE
	D2	Internal, silicone

Table 3: **96-way screw top cryogenic storage tubes from different suppliers were tested in a long-term storage test in the vapour phase of liquid nitrogen.**
Except for Greiner Bio-One all other tube manufacturers are anonymised and tube codes are utilised to differentiate between different tube types offered by some manufacturers (e.g. manufacturer 'A' offers two different tube types with different types of screw cap; 'A1' and 'A2').

4/ LONG-TERM STORAGE IN VAPOUR PHASE ABOVE LIQUID NITROGEN

4.1/ BACKGROUND

More and more biobanks store their samples in screw top tubes below -130 °C in the vapour phase of liquid nitrogen. Such ultra-low temperature storage is regarded as an optimum for best sample conservation[v vi vii], but also a technical challenge for the actual sample tube and its screw cap. Today, several tube types with different types of screw caps are offered **(figure 5)**, each promising a tight tube closure at ultra-low temperatures with only minimum amounts of water phenomenon observed at ultra-low temperatures and as such may cause changes in sample volume and biomarker concentration over time. Here 96-way tubes from different suppliers with different screw cap types were stored in the vapour phase above liquid nitrogen and tested for their ability to retain the original sample volume over time.

4.2/ METHOD

96-way cryogenic tubes from different suppliers **(see table 3)** were filled with water or serum (80 % of working volume suggested by supplier) and closed with a Hamilton LabElite Decapper at 6-7 Ncm (n=59) or manually at 5-6 Ncm (n=8). The tubes were subsequently weighed with a precision scale and stored in 96-way racks in the vapour phase of liquid nitrogen. Tubes were thawed overnight, thus avoiding any condensation of air moisture onto the tube's exterior, and subsequently weighed with a precision scale. Weighing was carried out at several time points.

4.3/ RESULTS AND CONCLUSION

Cryo.s Biobanking Tubes (300 µl and 1000 µl, abbreviated as 'G1' and 'G3') closed with a Hamilton LabElite Decapper at 6-7 Ncm revealed excellent tightness with less than 0.2 % loss of water after 550 days of storage in the vapour phase above liquid nitrogen **(figure 6A)**. In a second set of long-term storage tests, tubes from several tube suppliers were compared: The sample retention observed in this test after 152 days of storage ranged from 90.44 % to 99.94 % **(figure 6B)**. Whereas well sealing tubes were found within each category of tube closing systems (internal, silicone; internal, TPE and external, sealing lip), the gap between worst and best performers of each group was most narrow for silicone sealed tubes, followed by TPE sealed tubes, followed by external thread tubes with sealing lip and no additional flexible

GREINER BIO-ONE MANUFACTURES NON-STERILE 96-WAY CRYO.S BIOBANKING TUBES

which are produced under highest hygienic standards and which (although being referred to as 'non-sterile') are tested and certified free of detectable contaminants such as DNA, DNase, RNase, endotoxins and others. These tubes are recommended for the cryogenic storage of liquid samples. Sterile Cryo.s Biobanking Tubes, also part of Greiner Bio-One's product portfolio, are the first choice for the storage of viable cells for future cell culture or cell lysis with subsequent biomarker analysis

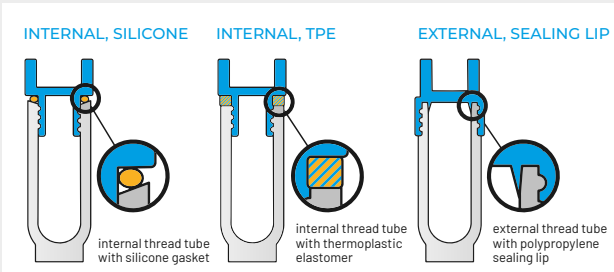

Figure 5: Types of cryogenic tubes
classified based on their type of screw cap and sealing material. Shown are cross sections through tubes (grey) with their screw cap on (blue) and the flexible sealing material, if present (orange or orange/blue).
TPE = thermoplastic elastomer.

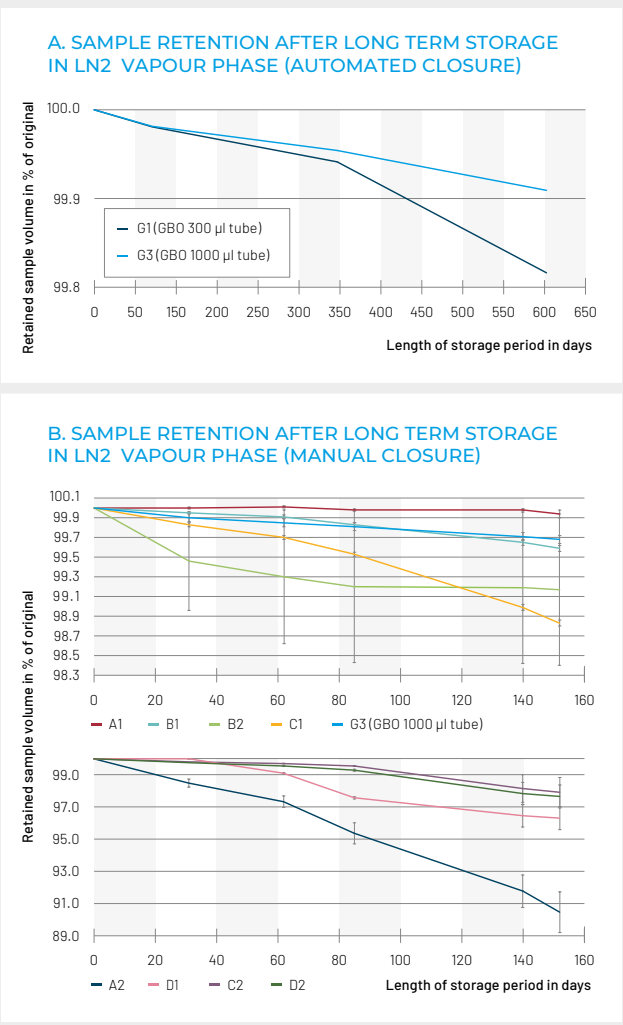

Figure 6: Sample retention after long-term storage in the vapour phase above liquid nitrogen. Tubes were closed with the Hamilton Labelite Decapper at 6-7 Ncm **(A)** or manually **(B)**. Error bars indicate standard errors of the mean.

GREINER BIO-ONE'S CRYO.S BIOBANKING TUBES

are excellently suited for the long-term storage of aqueous samples in the vapour phase above liquid nitrogen.
It is recommended to utilise semi-automated (e.g. Greiner Bio-One's eight-channel handheld decapper, item no. 852070) or fully automated devices (e.g. Hamilton's LabElite Decapper) for tube closure with a recommended torque value of 6-7 Ncm.

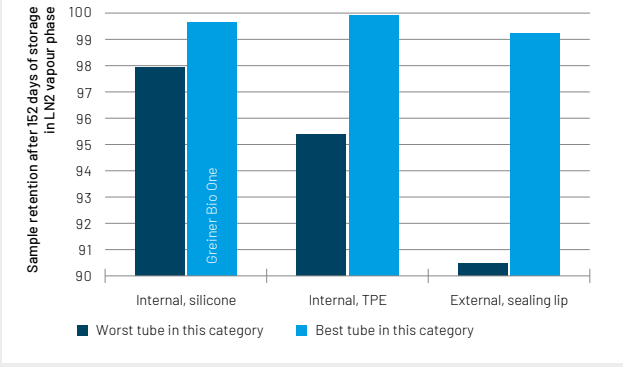

Figure 7: Performance range of different screw cap types in long-term vapour phase storage test.

sealing material[2] **(figure 7)**. In conclusion, the long-term storage test presented here revealed differences in the ability of different tubes to retain sample volume over time. These differences may not only be explained by differences in the type of tube closure; rather, other product features seem to exert influence on tube tightness. It is likely that parameters such as tube material, tube wall thickness and over-all tube design play an important role in long-term sample retention. The data shown within, however, indicate that the combination of internally threaded tubes with silicone gasket provide a very reliable and robust solution for long-term storage in the vapour phase above liquid nitrogen.

5/ DATAMATRIX QUALITY

5.1/ BACKGROUND

Machine-readable codes, in particular datamatrix codes, are state of the art solutions for an error-free, unambiguous and efficient identification and tracking of samples in biobanking. Most manufacturers of cryogenic sample storage tubes utilise laser technologies for applying datamatrix codes onto storage tubes. The major advantage of such

2 Note that at the time these studies were finalsed, externally threaded tubes with additional TPE sealing material were launched by some competitors which, because of their late availability, could not be included in this comparative study.

direct tube labelling over adhesive barcode labels is better temperature, mechanical and chemical resistance. Whereas adhesive labels bear the risk of detachment during long storage periods, with increased risk at cryogenic storage temperatures; laser etched labels persist both harsh storage conditions and long periods of time.

In principle two laser-labelling technologies are utilised for applying datamatrix codes onto storage tubes: **(1)** material foaming and **(2)** material removal. During material foaming, the laser beam melts the material. As a result, gas bubbles are produced in the material, which reflect the light diffusely. Thus, the laser mark is rendered lighter than all non-etched areas. This type of laser marking requires a dark (black) portion of the storage tube. Code application by material removal requires a part of the tube irreversibly coated with two contrasting thin layers of material. The outer material layer is dark (thus laser light absorbing) and completely vapourised after absorption of the laser light.

The second layer (usually white) reflects the laser, thus avoiding further material removal. This layer becomes visible as a white mark surrounded by the non-etched dark material. Both technologies produce datamatrix labels which have proven resistant against chemicals used in the context of laboratory and biobanking work, such as ethanol and isopropanol used for disinfection and DMSO used as a cryoprotectant in cell banking and as a solvent in compound storage.

Beside the chemical, thermal and mechanical stability of datamatrix codes on cryogenic storage tubes, the code print quality is an important quality criterion. A widely accepted methodology for characterising the print quality of datamatrix symbols is described in ISO/IEC 15415. In this standard several symbol properties, such as symbol contrast, axial nonuniformity and unused error correction, are delineated and their assessment under standard conditions (defined light source and aperture)[3] is described. The datamatrix symbol quality is important, as it directly influences the readability of the code, hence the identifiability of the datamatrix-carrying sample storage tube – the lower the quality of a datamatrix code, the higher the vulnerability of the code to mechanical damage and subsequent loss of readability.

THE DATAMATRIX CODE ON GREINER BIO-ONE'S CRYO.S BIOBANKING TUBES

is 'best in class' and provides a robust and reliable means for the machine readable identification of sample tubes in biobanking. A clear human readable text surrounding each datamatrix symbol helps to identify the datamatrix code content with the bare eye without requirement of a datamatrix reader.

5.2/ METHOD

Here we validated the quality of the datamatrix codes on cryogenic storage tubes from six different suppliers **(table 4)** using an Integra 9505 Barcode Quality Station from Label Vision Systems, Inc. Eight randomly selected tubes were analysed per supplier. In particular, the following code parameters were assessed.

- **Symbol contrast – detecting the differences between the dark and light areas as seen by the scanner,**
- **Modulation – measuring differences in contrast between adjacent areas of the datamatrix code,**
- **Axial nonuniformity – assessing uneven scaling of the datamatrix symbol along its X or Y axis,**
- **Unused Error Correction – measuring the reading safety margin that error correction provides [4],**

3 For details please refer to the standard 'ISO/ IEC 15415:2011 Information technology — Automatic ident fication and data capture techniques — Barcode symbol print quality test specification — Two-dimensional symbols'.

4 Unused error correction indicates the amount of available error correction in a symbol. Error correction is a method of reconstructing data that is lost via damage, erasure of the symbol, or poor printing. 100% unused error correction is the ideal case.

/ **Fixed Pattern Damage – analysing any damage to the finder pattern, quit zone and clocking pattern in the symbol (for explanation see figure 10, I).** All parameters were graded on a scale of 0 to 4 with 4 as the best possible result.

5.3/ RESULTS AND CONCLUSION

In the datamatrix validation test Greiner Bio One's Cryo.s Biobanking Tubes revealed outstanding results with excellent scores for symbol modulation, axial nonuniformity, non-used error correction and defects in finder pattern. The symbol contrast category yielded very good results, with remarkable extremely low variation between individual tubes. All other tube brands yielded inferior results **(figure 9)**. Some defects or problems identified with these tubes are illustrated in **figure 8**[II, III, IV].

6/ SUMMARY

Here we analysed the raw material purity, biomolecule adsorption, long-term tightness and datamatrix quality of cryogenic storage tubes from different suppliers. The data presented here shall sharpen the awareness for the sample storage tube as an important and potentially quality-determining component of biobanking. Greiner Bio-One's Cryo.s Biobanking Tubes yielded outstanding results in the individual tests and thus represent an optimum solution for cryogenic sample storage within the context of biobanking. **Table 5** summarises several suggested good practices for biobanking.

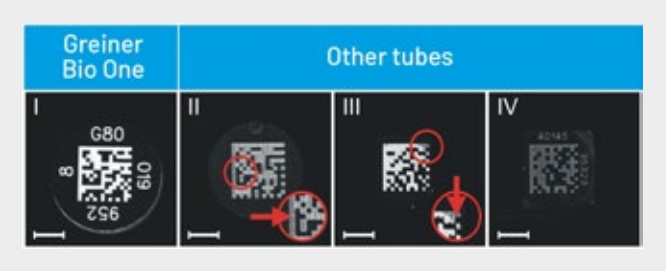

Figure 8: Examples of datamatrix codes on storage tubes.
I: Cryo.s Biobanking Tubes feature a 14 x 14 datamatrix code with surrounding human readable text. The L-shaped border (left/top) is the finder pattern. Together with the dashed line (clocking pattern) at the opposite margins of the symbol it helps to locate the symbol and determine its orientation. Each datamatrix code must be surrounded by a non-patterned area (quiet zone).

II, III: Datamatrix code examples with high modulation and defects in finder patterns (arrows).
IV: Datamatrix code with low contrast (scale bar = 2 mm).

Manufacturer Code	Tube Code	Datamatrix application	Size of datamatrix on tested tube type (dots)	Human readable text next to datamatrix code
Greiner Bio-One	G1 (Cryo.s Biobanking Tubes)	Laser	14 x 14	•
A	A1	Laser	14 x 14	-
B	B1	Laser	12 x 12	-
C	C1	Laser	12 x 12	•
D	D1	Laser	14 x 14	-
	D2	Laser	12 x 12	-

Table 4: 96-way cryogenic storage tubes from different suppliers were tested for datamatrix quality.
Except for Greiner Bio-One all other tube manufacturers are anonymised and tube codes are utilised to differentiate between different tube types offered by some manufacturers (e.g. manufacturer 'D' offers the two different tube types 'D1' and 'D2'). 14 x 14 datamatrix codes can encode tube IDs composed of 16 digits or 10 alphanumeric characters. Their intrinsic error correction tolerates 5-7 errors/erasures. 12 x 12 datamatrix codes can encode tube IDs composed of 10 digits or 6 alphanumeric characters. Their intrinsic error correction tolerates 3 errors/erasures.

Tube Type	Test tube No.	Symbol contrast	Modulation	Axial nonuniformity of datamatrix	Non-used error correction	Defects in finder pattern
G1 (Greiner Bio-One)	1	3	4	4	4	4
	2	3	4	4	4	4
	3	3	4	4	4	4
	4	3	4	4	4	4
	5	3	4	4	4	4
	6	3	4	4	4	4
	7	3	4	4	4	4
	8	3	4	4	4	4
A2	1	1	2	4	4	3
	2	1	2	4	4	2
	3	1	3	4	4	3
	4	1	1	4	4	3
	5	1	2	4	4	3
	6	1	2	4	4	2
	7	1	2	4	4	2
	8	1	2	4	4	3
B1	1	1	1	4	4	0
	2	1	1	4	4	0
	3	1	1	4	4	1
	4	1	1	4	4	0
	5	1	0	4	1	0
	6	1	1	4	4	0
	7	1	1	4	4	0
	8	1	0	4	4	0
C1	1	1	2	4	4	2
	2	1	4	4	4	3
	3	1	4	4	4	2
	4	1	2	4	4	2
	5	0	1	4	4	1
	6	1	2	4	4	4
	7	1	4	4	4	4
	8	1	2	4	4	3
D1	1	1	3	4	4	1
	2	2	3	4	4	1
	3	2	3	4	4	0
	4	2	0	4	4	0
	5	2	2	4	4	1
	6	1	2	4	4	1
	7	1	2	4	4	0
	8	1	2	4	4	1
D2	1	2	2	4	4	3
	2	2	4	4	4	1
	3	3	4	4	4	0
	4	2	4	4	4	3
	5	3	4	4	4	1
	6	2	4	4	4	3
	7	3	0	4	1	0
	8	2	4	4	4	1

Figure 9: Datamatrix quality of different sample tubes.
Symbol contrast, modulation, axial nonuniformity, nonused error correction and defects in the finder pattern are scored from 0 (bad performance) to 4 (best performance, no defects).

Aim	Solution
» Maintaining high cell viability, keeping cells mostly unaltered «	Store in the vapour phase of liquid nitrogen.
	Avoid or reduce periods of exposure to higher temperatures.
	Minimise time from sample acquisition until freezing.
	Use appropriate freezing medium (10-20 % DMSO, serum).
	Freeze at -1 K/min cooling rate.
	Avoid contamination of storage tube with cytotoxic substances.
	Avoid contamination of storage tube with endotoxins.
	Strictly avoid repeated freeze-thaw cycles.
» Avoiding biomarker disintegration in liquid sample (e.g. serum) «	Store at -80 °C or below.
	Avoid or reduce periods of exposure to higher temperatures.
	Minimise time from sample acquisition until freezing.
	Strictly avoid repeated freeze-thaw cycles.
» Avoiding changes of biomarker concentration in liquid sample «	Use storage tube which closes tightly at storage temperature.
	Avoid non-specific biomarker adsorption onto storage tube surface.
	Avoid contamination of storage tube with degrading enzymes (e.g. DNases, RNases).
» Avoiding sample contamination «	Use storage tube made of pure material, low in additives and leachables.

Table 5: **Good practice in biobanking:** Challenges and solutions.

7/ PRODUCT VERSIONS AND ORDERING INFORMATION

- **Choose between 235, 580 and 975 µl working volume**
- **Store 96 tubes per rack with ANSI/SLAS footprint in a very space efficient manner**
- **Bulk-packed tubes and customised codes on request**

Cryo.s Biobanking tubes are for storage of tissue, cells, fungi, bacteria, spores, cellular extracts or body fluids at ultra-low temperature for research and development purposes in a very compact format. Cryo.s Biobanking tubes must not be stored in the liquid phase of liquid nitrogen, but only in the gas phase above. Cryo.s Biobanking tubes are not intended for any application in the context of reproductive medicine.

Cryo.s Biobanking Tubes 300 µl

Height: 18.7 mm, Total rack height: 21.6 mm, Ø: 8.8 mm, Barcode type: datamatrix code, Raw material: PP, Working volume: ≤235 µl

Item no.	Barcode	Cap colour	Cap design	Thread type	Type of packaging	Sterile	Qty. inner / outer
976570	yes	natural	without screw cap	internal	rack		480 / 960
976580	yes	natural	screw cap	internal	rack		480 / 960
976561	yes	natural	screw cap	internal	rack	+	480 / 960
976586	yes	green	screw cap	internal	rack		480 / 960
976566	yes	green	screw cap	internal	rack	+	480 / 960
976585	yes	yellow	screw cap	internal	rack		480 / 960
976565	yes	yellow	screw cap	internal	rack	+	480 / 960
976584	yes	blue	screw cap	internal	rack		480 / 960
976564	yes	blue	screw cap	internal	rack	+	480 / 960
976583	yes	red	screw cap	internal	rack		480 / 960
976563	yes	red	screw cap	internal	rack	+	480 / 960
976588	yes	pink	screw cap	internal	rack		480 / 960
976568	yes	pink	screw cap	internal	rack	+	480 / 960
976589	yes	brown	screw cap	internal	rack		480 / 960
976569	yes	brown	screw cap	internal	rack	+	480 / 960
976587	yes	black	screw cap	internal	rack		480 / 960
976567	yes	black	screw cap	internal	rack	+	480 / 960
131202	yes	natural	screw cap	internal	bulk		480 / 960
131263	yes	natural	screw cap	internal	bulk	+	480 / 960

- Follow the instructions of use provided within each box.
- USP class VI certified medical grade polypropylene

Cryo.s Biobanking Tubes 600 µl

Height: 33.3 mm, Total rack height: 36.2 mm, Ø: 8.8 mm, Barcode type: datamatrix code, Raw material: PP, Working volume: ≤580 µl

Item no.	Barcode	Cap colour	Cap design	Thread type	Type of packaging	Sterile	Qty. inner / outer
977570	yes		without screw cap	internal	rack		192 / 960
977580	yes	natural	screw cap	internal	rack		192 / 960
977561	yes	natural	screw cap	internal	rack	+	192 / 960
977586	yes	green	screw cap	internal	rack		192 / 960
977566	yes	green	screw cap	internal	rack	+	192 / 960
977585	yes	yellow	screw cap	internal	rack		192 / 960
977565	yes	yellow	screw cap	internal	rack	+	192 / 960
977584	yes	blue	screw cap	internal	rack		192 / 960
977564	yes	blue	screw cap	internal	rack	+	192 / 960
977583	yes	red	screw cap	internal	rack		192 / 960
977563	yes	red	screw cap	internal	rack	+	192 / 960
977588	yes	pink	screw cap	internal	rack		192 / 960
977568	yes	pink	screw cap	internal	rack	+	192 / 960
977589	yes	brown	screw cap	internal	rack		192 / 960
977569	yes	brown	screw cap	internal	rack	+	192 / 960
977587	yes	black	screw cap	internal	rack		192 / 960
977567	yes	black	screw cap	internal	rack	+	192 / 960
132202	yes	natural	screw cap	internal	bulk		192 / 960
132263	yes	natural	screw cap	internal	bulk	+	192 / 960

- Follow the instructions of use provided within each box.
- USP class VI certified medical grade polypropylene

Cryo.s Biobanking Tubes 1000 µl

Height: 50.8 mm, Total rack height: 53.9 mm, Ø: 8.8 mm, Barcode type: datamatrix code, Raw material: PP, Working volume: ≤975 µl

Item no.	Barcode	Cap colour	Cap design	Thread type	Type of packaging	Sterile	Qty. inner / outer
978570	yes		without screw cap	internal	rack		192 / 960
978580	yes	natural	screw cap	internal	rack		192 / 960
978561	yes	natural	screw cap	internal	rack	+	192 / 960
978586	yes	green	screw cap	internal	rack		192 / 960
978566	yes	green	screw cap	internal	rack	+	192 / 960
978585	yes	yellow	screw cap	internal	rack		192 / 960
978565	yes	yellow	screw cap	internal	rack	+	192 / 960
978584	yes	blue	screw cap	internal	rack		192 / 960
978564	yes	blue	screw cap	internal	rack	+	192 / 960
978583	yes	red	screw cap	internal	rack		192 / 960
978563	yes	red	screw cap	internal	rack	+	192 / 960
978588	yes	pink	screw cap	internal	rack		192 / 960
978568	yes	pink	screw cap	internal	rack	+	192 / 960
978589	yes	brown	screw cap	internal	rack		192 / 960
978569	yes	brown	screw cap	internal	rack	+	192 / 960
978587	yes	black	screw cap	internal	rack		192 / 960
978567	yes	black	screw cap	internal	rack	+	192 / 960
133202	yes	natural	screw cap	internal	bulk		192 / 960
133263	yes	natural	screw cap	internal	bulk	+	192 / 960

/ Follow the instructions of use provided within each box.
/ USP class VI certified medical grade polypropylene

Screw Caps / Biobanking Tubes

Description: 96 screw caps in cap carrier, Raw material: PP, Cap design: screw cap

Item no.	Barcode	Cap colour	Qty. inner / outer
385270	no	natural	960 / 960
385276	no	green	960 / 960
385275	no	yellow	960 / 960
385274	no	blue	960 / 960
385273	no	red	960 / 960
385278	no	pink	960 / 960
385279	no	brown	960 / 960
385277	no	black	960 / 960

/ Follow the instructions of use provided within each box.
/ USP class VI certified medical grade polypropylene

96-way Datamatrix Cryo Rack

Barcode type: linear barcode and datamatrix code, Raw material: PC

Item no.	Height	Barcode	Rack colour	Suitable for Cryo.s	Qty. inner / outer
976501	19.1 mm	yes	● black	300 µl	5 / 10
977501	33.7 mm	yes	● black	600 µl	2 / 10
978501	51.4 mm	yes	● black	1000 µl	2 / 10

- / Follow the instructions of use provided within each box.
- / USP class VI certified medical grade polypropylene

All tests described in this document were performed applying good practice and maximum care. Competitor tubes analysed within represent the design status as commercially available in December 2015 or prior. Note since that time tube materials and designs of competitor products included in these tests may have been subject to change.

6/ LITERATURE

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Greiner Bio-One GmbH Frickenhausen, Germany
PHONE +49 7022 948-0 / **FAX** +49 7022 948-514 / **E-MAIL** info@de.gbo.com
Greiner Bio-One is a global player.
Find the contact details of your local partner on our website.

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