Biobanking

Edited by: M. Kiehntopf

Astrid Petersmann, Theresa Winter, Sophia Lamp and Matthias Nauck*

Relevant criteria for the selection of cryotubes. Experiences from the German National Cohort

<https://doi.org/10.1515/labmed-2019-0172>

Received October 8, 2019; accepted November 6, 2019; previously published online November 29, 2019

Abstract

Background: The storage of different biomaterials over long time periods is one of the main requirements of biobanking ensuring that modifications in the composition or any other change of the biomaterials have to be avoided. In the German National Cohort samples from around 200,000 participants are processed and stored long term.

Methods: A tender for cryotubes and racks was performed in 2013 setting up several characteristics that were judged against each other. Tubes and racks were evaluated regarding the performance and handling in connection with the main biorepository. With a 5-year experience using the selected tubes we are able to reflect some of the criteria of the tender.

Results: At the end of the decision, the former company FluidX, in the meantime taken over from Brooks (Brooks Life Sciences, Manchester, UK), received the order. The experience with the external testing of the tube was useful.

Conclusions: Overall, the experience with the cryotubes is good and their mechanical handling at the different sites is routine in the meantime. There are some aspects that we recommend for future tenders. Further research is necessary to learn more about the cryotubes and the labware in general in the field of biobanking to store our samples as safely as possible.

Keywords: automatization; biobanking; cryotubes; German National Cohort; tender.

Introduction

The storage of different biomaterials over long time periods – up to decades – is one of the main requirements of biobanking. The aim of the storage has to be that modifications in the composition or any other changes of the biomaterials have to be avoided. Therefore, one of the approaches to achieve this goal is the storage at low temperatures, down to around -195°C in the gas phase of liquid nitrogen. These low temperatures have positive effects in view of the sample quality, but the applied container for the biomaterials needs specific properties. In 2013 the German National Cohort (GNC) performed a tender for the cryotubes of the GNC. Within the GNC around 200,000 participants were recruited in the meantime in 18 study centres around Germany [1]. For each of the participants more than 100 aliquots were filled with biosamples at the basis recruitment. So over all, more than 20,000,000 cryotubes were used in this huge epidemiological study [2].

The biomaterials of the GNC participants were collected and processed locally employing highly standardized procedures in each of the 18 study centres. Processing of blood and urine samples started with the centrifugation of serum, ethylenediaminetetraacetic acid (EDTA) blood and urine. Subsequently, the primary tubes were placed on a Hamilton pipetting robot (Hamilton Germany GmbH – Robotics, Gräfelfing, Germany). The pipetting robot aliquoted the content of the primary tubes in several aliquots with a volume of 250 μL each:

***Correspondence: Prof. Dr. med. Matthias Nauck**, Integrated Research Biobank Greifswald, University Medicine Greifswald, Greifswald, Germany; Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany; and DZHK (German Centre for Cardiovascular Research), Partner Site Greifswald, University Medicine Greifswald, Greifswald, Germany, Phone: 0049 3834 865500, Fax: 0049 3834 865502, E-Mail: matthias.nauck@med.uni-greifswald.de

Astrid Petersmann: Integrated Research Biobank Greifswald, University Medicine Greifswald, Greifswald, Germany; Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany; and Institute of Clinical Chemistry, University Medicine Göttingen, Göttingen, Germany

Theresa Winter: Integrated Research Biobank Greifswald, University Medicine Greifswald, Greifswald, Germany; and Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany

Sophia Lamp: Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany

30 serum aliquots, 48 EDTA-plasma aliquots, six erythrocyte aliquots and 12 urine aliquots. In addition, aliquots of 500 µL were generated: one serum, four urine and three buffy coat aliquots.

All racks with the FluidX (Brooks Life Sciences [formerly FluidX], Manchester, UK) tubes were scanned using the Ziath flatbed (Ziath Ltd., Cambridge, UK) scanner. The FluidX tubes were opened and closed semi-automatically applying a capper/decapper from Hamilton.

Two-thirds of these aliquot samples were transported to a central biorepository at the Helmholtz-Zentrum near Munich, where most samples are now stored at -195 °C. One-third of the aliquots are stored peripherally in or around the recruiting study centres. In these peripheral biorepositories the storage temperatures are heterogeneous and are at least at -80 °C, but at other sites the storage temperature is -195 °C as well.

In the meantime, we have a 5-year experience with the selected tubes and therefore we are able to reflect some of the criteria of the tender.

Materials and methods

To identify the most suitable products the tender was structured into sections: general requirements, price as well as quality and performance-related requirements.

In the tender, at the beginning general requirements were defined, which are given in Table 1.

Table 2 shows the aspects of the original tender from 2013 with the category price playing the main role. The assessment criteria are listed in the table.

The main focus of the tender was the description and classification of the quality and performance criteria for the cryotubes. Therefore, many aspects were taken into account to avoid choosing cryotubes which could negatively influence the results of the GNC.

The GNC is the biggest German epidemiological study and the high quality standards of the whole study are one of its outstanding features. In view of biobanking, the automated processes of the samples play an important role over the next decades and therefore the handling of the labware was one of the quality criteria. Because the combination of the specific biorepository and the applied labware plays an important role, the manufacturers were asked to send their cryotubes and racks to the GNC. Using these cryotubes and racks provided by the manufacturer, the performance and handling in connection with the main biorepository was evaluated.

For this the following processes were performed and used for the evaluation:

Table 1: General requirements.

Specifications of the cryotubes
Suitability for the storage in the gas phase of liquid nitrogen, directly above the liquid nitrogen, which means at -195 °C. Please attach certificates
Sealable with screw cap
Maximal working volume between 190 and 300 µL (lot 1) and between 500 and 700 µL (lot 2) at ambient temperature
Working volume determination has to take into account the volume extension during freezing. Overpressure in the sealed tube must not occur. Furthermore, enough space has to be allowed to ensure that the inner lower end of the screw cap does not touch the fresh nor the frozen sample. This accounts for all parts of the screw cap, centre to side parts. The integration of a possible cavity in the screw cap is not sufficient. Contact of fresh or frozen biomaterial must not occur to any part of the screw cap
Fixed and irremovable 2D-code at the bottom of the tube and carrier of a GNC specific number range
Identical material of tube and screw cap. Sealing rings are excluded
Free of endotoxins, DNA, DNAses, RNAses, heavy metals
Tightness during long time storage about many years at -195 °C
Form stability of the tubes during long term storage for many years at -195 °C
Form stability of the caps during long term storage for many years at -195 °C
Form stability of the racks during long term storage for many years at -195 °C
Delivery of the cryotubes in SBS-racks with GNC specific 2D barcodes at the bottom of the rack
Capping and decapping of the cryo tubes must be possible with a commercially available capper/decapper (device MR-0710-01 of the company Hamilton)

Table 2: Prices.

a.1	Price for 96 cryotubes within the SBS-rack, unsterile	16 Points for the cheapest tenderer
a.2	Price for 96 twist-off caps in a tray, unsterile	12 Points for the cheapest tenderer
a.3	Price for an empty SBS-rack with cap	2 Points for the cheapest tenderer

Storage

- Using ten racks per cryotube type.
- The cryotubes were filled with the specified working volume.
- The caps were closed with the specified torque.
- The cryotubes were stored in the racks for at least 24 h at -80 °C.
- The cooled racks were stored for at least 48 h at -195 °C.

Table 3: Performance/quality (manufacturer).

b.1	Performance/quality	Tender: maximal 66 points possible
b.1.1	Which material are the cryotubes and screw caps composed of?	Polypropylene = 2 points Other materials = 0 points
b.1.2	Are investigations available to prove that there is no time dependent release of components of the cryotube into the sample liquid?	Yes = 2 points No = 0 points
b.1.3	Is the cryotubes' thread an internal or external thread?	External thread = 2 points Internal thread = 0 points
b.1.4	Type and material of the tightening between cryotube and screw cap	Sealing without O-ring = 2 points Sealing with O-ring = 0 points
b.1.5	Size of the caps' grip surface	>5 mm: 10 points 2–5 mm: 5 points <2 mm: 0 points
b.1.6	How is the 2D code fixed at the cryotube?	Via laser = 4 points Painted = 2 points Glued = 0 points
b.1.7	Is the 2D barcode (identifier) defined in a way that mix-ups are excluded?	Yes = 2 points No = 0 points
b.1.8	Is it possible to fix an additional, identical 2D code at the side of the cryotube?	Yes (via laser) = 4 points Yes (painted) = 2 points
b.1.9	Storage efficiency	Please give the dimensions of length (L) of SBS-rack $\times$ Width (W) of SBS-rack $\times$ Height (H) (H = distance between lower end of the rack and upper end of the screw cap) in cm, including two positions after the decimal point Lowest storage efficiency per working volume receives 0 points, best storage efficiency receives 20 points
b.1.10	Does a verification procedure exist to define the cryotubes' purity in view of being free of endotoxins, DNA, DNAses, RNAses, heavy metals?	Yes = 2 points No = 0 points
b.1.11	Is any data available to verify the tightness during long term storage at -195°C and comparable cryotubes?	Valid data according to storage longer than 10 years: 3 points Valid data according to storage between 5 and 10 years: 2 points Valid data according to storage up to 5 years: 1 point No data available: 0 points
b.1.12	Does the cryotubes' production occur in conditions, which guarantee the level of dust-free rooms (clean room)?	Yes = 5 points No = 0 points
b.1.13	Are the racks for central and peripheral storage available in two different colours?	Yes = 2 points No = 0 points
b.1.14	Has the fracture strength of the cooled cryotubes been tested by the manufacturer (stress test)?	Yes = 2 points No = 0 points
b.1.15	Was the fracture strength of the cooled racks tested by the manufacturer (stress test)?	Yes = 2 points No = 0 points
b.1.16	Will protocols about the production process be provided containing lot numbers, which document the quality assurance of the production?	Yes = 2 points No = 0 points

Table 4: Performance/quality (external testing).

b.2	Performance/quality	External testing: 52 points possible
b.2.1	Does the racks' form keep unchanged?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)
b.2.2.	Does the racks' planarity keep unchanged?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)
b.2.3	Does the racks' mechanical stability persist?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)
b.2.4	Does the centring aid remain unchanged?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)
b.2.5	Do the 1D and 2D barcodes on the racks remain fixed and readable?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)
b.2.6	Does the cryotubes' outer form remain unchanged?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)
b.2.7	Does the racks' mechanical stability persist?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)
b.2.8	Does the self-centring remain unchanged?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)
b.2.9	Is an accidental tilt of the cap excluded?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)
b.2.10	Does the thread remain unchanged?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)
b.2.11	Do the 1D and 2D barcodes on the cryotubes remain fixed and readable?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)
b.2.12	Is the working volume specified correctly?	Yes = 2 points No = 0 points
b.2.13	Is the specification of the caps' torque correct?	Yes = 2 points No = 0 points
b.2.14	According to the error rate at the picking test the functionality of the cryotubes will be judged in view of automatization:	The product with the lowest error rate receives 20 points. Zero points receives the product with the highest error rate. The points for the other products are calculated according to linear interpolation
b.2.15	Is the maximal stated height of the cryotubes' caps always met?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)
b.2.16	Do the cryotubes stay fixed in the rack during decapping and capping?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)
b.2.17	Can formation of ice on the cryotubes or racks be observed?	Yes: 2 points No, small changes (functionality given): 1 point No, relevant changes (functionality affected)

Table 5: Delivery.

C	Delivery	24 Points possible
c.1	Time between an order placed by a study centre or member of the GNC and delivery (regulatory calls of the 18 study centres/members of the GNC)	Within 1 week = 4 points 1 week to 1 month = 2 points >1 month = 0 points
c.2	To which time point is the delivery of 1 million cryotubes possible?	Delivery after the acceptance of the tender: Within 1 month: 20 points Within 2 months: 10 points Within 3 months: 5 points more than 3 months: 0 points

Picking

- Four out of ten racks were removed from the biorepository. If the racks were deformed, the most deformed racks are applied for the next examinations.
- The cryotubes out of two racks were removed.
- An empty and a filled rack were placed in the picking unit at -80°C .
- All cryotubes were transferred from the filled rack into the empty rack.
- Afterwards these steps were repeated three times.
- In addition, the whole examination was repeated using two other racks.

Capping of cryotubes

- The defined torque was exceeded with the intention to simulate a handling error.
- The cryotubes of two racks were capped and decapped several times to get an impression of this mechanical process.

The criteria of these external testing programs are listed in Table 4.

The delivery of such a high number of cryotubes was mandatory for the GNC, especially at the starting period.

Table 6: Additional product information without relevance for the judgement of the tender.

d.1	If it exists: which kind of examinations according to the delivery of components of the cryotubes material into the samples are available?
d.2	If it exists: which possibility is given to place an additional, identical 2D barcode at the side of the cryotubes?
d.3	If it exists: according to which criteria was the fracture strength of the cooled cryotubes tested by the manufacturer (stress test)?
d.4	If it exists: according to which criteria was the fracture strength of the cooled racks tested by the manufacturer (stress test)?

Therefore, delivery criteria were judged as well using Table 5.

In addition to the already mentioned criteria listed in Tables 1–5 the manufacturer had the possibility to describe specific quality aspects in detail in order to emphasize the quality of their products (Table 6). However, these answers did not play any role in the classification of the products.

As the conducted research is not related to either human or animal use ethical approval was not needed.

Results

Several companies applied for the tender. At the end of the decision, the former company FluidX, in the meantime taken over from Brooks, received the order. The decision was made for a – at that time – brand-new cryotube, consisting of the tube itself and a black jacket around. This jacket allowed to place a 2D code both at the bottom of the tube and, additionally, an identical 2D code at the side of the tube. This increases safety, because in case of damage for any reason of one of the 2D barcodes the other 2D barcode still allows the identification of the tube. In addition, the possibility to read a 2D barcode at the side of the tubes allows the laboratory to perform analyses of these tubes in instruments, which are not capable of reading the 2D code at the bottom of the tube.

The use of cryotubes with external screw caps allowed an efficient storage of the collected biomaterials.

The experience with the external testing of the tube was quite useful as, for instance, some of the manufacturers did not document the correct working volume. These errors could be clarified during the testing period.

The availability of the tubes was a challenge at the beginning of the GNC study, because many tubes were needed in parallel and the production of these tubes had to be increased.

Discussion

The GNC finished the recruitment of the basis examination in the meantime. Therefore, we have experience within the GNC with the handling of more than 20,000,000 cryotubes.

Overall, the experience with the cryotubes is good and the mechanical handling of the cryotubes at the different sites is routine in the meantime. The devices, like the pipetting robot or the capper/decapper, perform well within the GNC. However, despite the high work load, the processes are highly standardized and, therefore, the variability in daily routine work is small. This is one of the benefits of such a study, using its own devices and have their own skilled personnel.

Until now, we do not have any negative experiences according to the tightness of the cryotubes. As so far no analytical measurements have been performed there are no experiences regarding possible contaminations of the biosamples caused by components of the cryotubes. Here we will get additional information when we start to analyse these samples with highly sensitive methods.

In view of tenders in the future there are some aspects we recommend to take into account:

- Correct specification of the working volume.
- Specific margins for tolerance limits should be defined, for example the torque.
- The external testing of the labware handling should include all affected laboratory devices, like pipetting robots, capper/decapper, picking modules and the biorepositories themselves.
- Lot-to-lot variation in view of all important properties of the cryotubes.
- Tests to identify interactions of tube materials with biological substances (e.g. hydrophobic and hydrophilic) should be developed and investigated.
- Criteria for test scenarios in view of leakage and permeability in long-term studies should be developed.
- Consideration of specific needs and workflows, e.g. a high degree of automation may need more intensive testing of the offered material.

The tender for the cryotubes for the GNC represents a comprehensive state-of-the-art summary of important aspects even 6 years after the decision criteria were defined. To build a basis for future studies working groups with experts from different biobanks and studies should summarise experiences from other tenders and make it available for the biobanking community. Also, the first quality assessment tools have been developed [3–6] and quality markers have been studied [7, 8] in order to secure the high

quality of the bio material. Important to note: the components of the cryotubes itself were not considered in these publications at all. However, the labware itself can play an important role in the field of biobanking and, therefore, important criteria should be considered in future to a stronger extent in comparable tenders.

It is important for the biobanking community to be aware of changing materials and production techniques, e.g. co-molding, in order to improve the products. Also, commonly accepted standard procedures for testing need to be developed, for example for providing valid and comparable data on the tightness of cryotubes. Therefore, independent research needs to accompany these product changes to learn more about the cryotubes and the labware in general in the field of biobanking to store our samples as safely as possible.

The tender of the GNC specified several aspects of the labware. However, each study and each biobanking environment has its own needs, and therefore, specific items will be important for the decision made in each biobank. The storage time can vary from some days to decades, which has influence on several aspects of the cryotubes. The sample size has to be taken into account and the manual or more or less automated way of sample processing. The kind of the codes might be important in view of the analyses and the platforms, which will be used in the future. From this point of view, the type of codes, the labelling at the bottom or the side, the persistence of the codes against chemical and other detergents might be important, depending on the given environment, where the samples were collected and stored. Therefore, each tender needs to be carefully adjusted to the needs of the individual biobank and study.

In summary, one important goal of this publication is the aspect that the price of the products alone should not define the decision for or against a cryotube. There are several more aspects, which can play an important role in view of the sample quality and the value of the scientific research based on samples of the biobanks.

Author contributions: All the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Research funding: None declared.

Employment or leadership: None declared.

Honorarium: None declared.

Competing interests: The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

References

1. National Cohort. Die NAKO-Gesundheitsstudie geht in die zweite Runde, 2019. Available at: <https://nako.de/blog/2019/05/03/die-nako-gesundheitsstudie-geht-in-die-zweite-runde/>. Accessed: 7 Oct 2019.
2. Wichmann HE, Hörlein A, Ahrens W, Nauck M. The biobank of the German National Cohort as a resource for epidemiologic research. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 2016;59:351–60.
3. Gaignaux A, Ashton G, Coppola D, De Souza Y, De Wilde A, Eliason J, et al. A biospecimen proficiency testing program for biobank accreditation: four years of experience. *Biopreserv Biobank* 2016;14:429–39.
4. Betsou F, Gunter E, Clements J, DeSouza Y, Goddard KA, Guadagni F, et al. Identification of evidence-based biospecimen quality-control tools: a report of the International Society for Biological and Environmental Repositories (ISBER) Biospecimen Science Working Group. *J Mol Diagn* 2013;15:3–16.
5. Kirwan JA, Brennan L, Broadhurst D, Fiehn O, Cascante M, Dunn WB, et al. Preanalytical processing and biobanking procedures of biological samples for metabolomics research: a white paper, community perspective (for “Precision Medicine and Pharmacometabolomics Task Group”-The Metabolomics Society Initiative). *Clin Chem* 2018;64:1158–82.
6. Hubel A, Aksan A, Skubitz AP, Wendt C, Zhong X. State of the art in preservation of fluid biospecimens. *Biopreserv Biobank* 2011;9:237–44.
7. Kang HJ, Jeon SY, Park JS, Yun JY, Kil HN, Hong WK, et al. Identification of clinical biomarkers for pre-analytical quality control of blood samples. *Biopreserv Biobank* 2013;11:94–100.
8. Malm L, Tybring G, Moritz T, Landin B, Galli J. Metabolomic quality assessment of EDTA plasma and serum samples. *Biopreserv Biobank* 2016;14:416–23.