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TechNote

LVL SAFE® 2D Tubes and racks – quality for long-term sample integrity

Verified suitability for long-term storage, transport, and automated sample handling

The secure and reproducible storage of sensitive samples – whether biological, chemical, or otherwise analytically relevant – places high demands on sample containers, especially under extreme temperatures and within automated processes. LVL SAFE® 2D Tubes were developed for these diverse applications: from long-term cryogenic storage in biobanks to routine use in research, diagnostics, and production.

This technical documentation provides comprehensive test results to confirm the essential quality characteristics – including leak-tightness and long-term mechanical stability, chemical inertness, low binding, biocompatibility, and suitability for automation. All tests were conducted and documented in accordance with established standards (including DIN 13279-2022-05, ISO 20070, ISO 10993, ISO 11137). The aim is to equip users with the necessary information and criteria to make well-informed decisions when selecting high-quality sample containers suitable for their specific applications.

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QUALITY PROFILE AT A GLANCE: ALL TEST CRITERIA AND RESULTS

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Relevant standards and guidelines considered

The relevant standards for biobank sample containers, including DIN 13279-2022-05, ISO 20070, and IATA regulations, were taken into account during the design of the tubes and the selection of testing methods. These standards are systematically addressed in the respective chapters.

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Optimized material selection and tube design

LVL SAFE® 2D Tubes are made of a specially developed, low-leachable polypropylene featuring defined material purity and structural stability. The polymer matrix is homogeneous, completely additive-free, and contains no co-monomers – an essential quality feature ensuring maximum inertness and sample compatibility. The combination of precisely molded tube geometry, an integrated TPE seal produced via two-component molding, and permanently laser-engraved 2D codes is purpose-built for use in cryogenic environments and automated workflows.

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Leak-tightness ensured – resulting long-term stability

The innovative product design provides the foundation for the outstanding leak-tightness of LVL SAFE® 2D Tubes. Extensive testing – including the gravimetric leak test in accordance with IATA, CO_2 leak testing, nitrogen permeation testing at −180°C, and repeated open/close cycle leak tests – demonstrates consistently reliable sealing performance. Even under mechanical stress, such as up to 15 closure cycles or centrifugation up to 10,000 × g (for individual tubes), the tubes remain tightly sealed and functionally stable.

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Maximum reliability in automation and robotics

LVL SAFE® 2D Tubes, caps, and racks meet all requirements for reliable use in automated storage, liquid handling, and sample-processing systems. Geometry, stability, and manufacturing tolerances are fully SBS-compatible and designed for robotic integration. LVL SAFE® 2D Tubes demonstrate consistent compatibility with automated storage systems from leading manufacturers. Suitability for automated storage logistics – including precise tube picking, secure positioning, and reliable barcode recognition – has been validated through manufacturer-specific testing protocols. Successful test outcomes are supported by official compatibility certifications. Particularly noteworthy is the high quality of the coding: in testing according to ISO 15415 and ISO 15416, LVL SAFE® 2D Tubes were the only product to consistently achieve top scores in all evaluation categories – including contrast, error correction, readability, and quiet zone. Additional practical functionality tests – including capping/decapping, pick & place handling, and rack stability under load – also confirmed the process safety and robustness of the LVL SAFE® 2D Tubes.

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Proven chemical inertness – outstanding compared to market products

Chemical inertness is critical to maintaining sample quality and ensuring the long-term performance of cryobanking tubes. Extensive testing of the LVL SAFE® 2D Tubes has shown that no migratable substances are released in relevant concentrations. Neither broad-spectrum leachables and extractables screening (GC-MS, LC-MS) nor targeted analyses of plastic-related processing residues detected any relevant substances. In addition, the absence of heavy metals was confirmed. Compared to two anonymized market products, LVL SAFE® 2D Tubes consistently showed lower or non-detectable signal intensities, indicating exceptionally high material purity.

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Low binding – verified for proteins and nucleic acids, no degradation

The SAFE® PP material was specifically developed to prevent the adsorption of proteins or nucleic acids onto container walls, thereby avoiding sample loss in biological matrices. Extensive studies have demonstrated that sample integrity in this regard is exceptionally well maintained. LVL SAFE® 2D Tubes exhibit excellent low binding properties for both proteins and nucleic acids. Independent studies conducted by university research laboratories specialized in proteins and nucleic acids revealed significantly lower losses of proteins and nucleic acids compared to comparison products – including tubes with declared low-binding coatings. Sample integrity was fully preserved in all tests. Both mass spectrometry analyses for proteins and qPCR-based stability tests for DNA confirmed the high material quality and minimal surface binding.

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Verified and certified biological purity and biocompatibility

Certified laboratories have confirmed that LVL SAFE® 2D Tubes are free from pyrogens, DNase, RNase, human DNA contamination, endotoxins, mycoplasma, BSE/TSE agents, and animal-derived raw materials. The tubes have passed and been certified for biocompatibility and cytotoxicity testing.

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Sustainability – reduced energy consumption and CO_2 emissions, recyclable material

Now more than ever, cryobanking tubes must not only be functional but also environmentally friendly. With LVL SAFE® 2D Tubes, space requirements, energy consumption, and CO_2 emissions can be reduced by up to 80% compared to standard tubes. Tube racks can be recycled and reused.

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Evaluation summary

LVL SAFE® 2D Tubes demonstrate consistently high quality across all tested areas and meet all applicable standards. In comparative studies of automation compatibility, chemical inertness, and low binding properties, they outperformed other commonly available products. The results confirm their outstanding suitability for long-term storage, secure sample transport, and use in diagnostics and automated laboratory and storage systems.

DIN 13279-2022-05 / ISO 20070 – requirements for sample containers for the storage of biological materials in biobanks

The DIN 13279 standard defines requirements and guidelines for the storage and handling of biological samples in biotechnology. It ensures that sample quality, safety, and traceability are maintained through standardized processes. The standard includes specifications for sample collection, preparation, storage, and preservation. Particular emphasis is placed on the use of cryogenic storage methods and automated storage systems to preserve the integrity of biological samples over the long term.

General requirements

The standard specifies general requirements for the materials, design, and manufacturing of sample containers. It mandates that containers must be made of materials that are biocompatible and chemically inert to prevent contamination.

Leak-tightness

Sample containers must be completely sealed to prevent sample leakage. They must be able to withstand various pressure and temperature fluctuations without becoming compromised.

Temperature resistance

Containers must be capable of enduring extreme temperatures, including very low temperatures during cryopreservation (down to −196°C) and high temperatures during sterilization processes. Specific testing must be conducted to ensure that the containers can withstand these conditions without material failure or deformation.

Mechanical strength

Containers must be mechanically stable and must not crack or break under normal handling and storage conditions. Mechanical stress tests are conducted to assess the strength and fracture resistance of the containers.

Compatibility with automated systems

Containers must be designed to be compatible with automated liquid handling and storage robotics systems. Requirements are specified for the dimensions and shape of the containers to ensure seamless integration into such systems.

Labeling and traceability

Sample containers must be clearly labeled to allow easy identification and traceability. The standard specifies that containers should be equipped with machine-readable codes (e.g., barcodes or 2D codes) to enhance efficiency and accuracy throughout the biobanking process.

Testing and verification methods

The standard describes detailed testing procedures that must be used to verify compliance with the above requirements. These include physical, chemical, and mechanical tests, as well as tests for leak-tightness and temperature resistance.

PRODUCT FUNDAMENTALS AS A QUALITY FOUNDATION

Polymer selection – SAFE® L3 Medical Polypropylene

LVL SAFE® 2D Tubes are made using a specially developed polypropylene plastic. This polymer, formulated exclusively for LVL, is precisely tailored to meet the application-specific requirements of cryogenic tubes. It is a virgin, medical-grade polypropylene compliant with USP Class VI.

The composition of the material has a significant impact on both quality and achievable performance levels – an aspect to which we have dedicated particular attention. This is reflected in the designation "SAFE® L3 Medical Polypropylene," which highlights three key material properties:

L3 stands for:

- low leachables
- low binding (minimal biomolecular adsorption)
- low permeability

Achieving these and other critical properties is the result of years of intensive research conducted in collaboration with leading global polymer manufacturers.

We have come to understand that sample tubes require a maximally pure polymer structure – one that is free from additives such as flow enhancers, anti-stick agents, or mold release compounds. The logic is simple:

"Anything added to the polymer and incorporated into the base matrix can – especially over time – leach out again."

This insight led us to the conclusion that only a highly homogeneous, closed polymer matrix can produce a plastic with high inertness. The absence of potential "attachment points" prevents dipole interactions, resulting in minimal interactions between the sample and the tube body. At the same time, the homogeneous structure enables particularly dense packing of polymer chains, which becomes especially beneficial in cryogenic environments due to extremely low permeability.

A homogeneous, intact polymer matrix minimizes reactive surface areas and thus reduces binding affinity for polar substances. It also effectively prevents the migration of components through leaching – even when in contact with solvents.

For this reason, we consistently refrain from using additives or blending foreign monomers. These could lead to an undefined and thus non-reproducible polymer structure – as illustrated in the schematic diagram below.

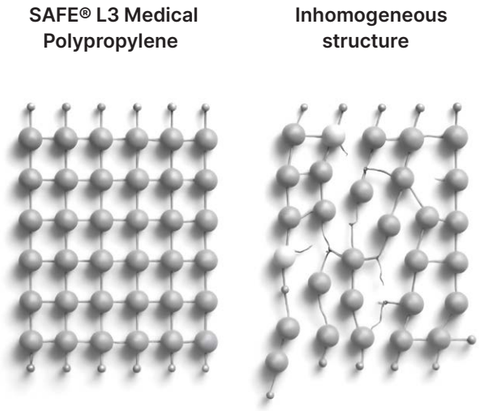


Fig. 1: Symbolic illustration of a homogeneous (left) vs. inhomogeneous (right) polymer structure – for conceptual comparison only; not a chemical structure formula

Tube and closure design and functionality

Details on tube construction

The application-focused design of the LVL SAFE® 2D Tube represents another key pillar of the product's overall quality. The tube is manufactured using a multicomponent injection molding process involving high-precision molding tools.

In the first production step, a transparent, ultra-pure sleeve (made from SAFE® L3 Medical Polypropylene) is injection-molded. This sleeve is completely enclosed – except for the opening – and serves as the only component that comes into direct contact with the sample during use, as illustrated in the graphic below.

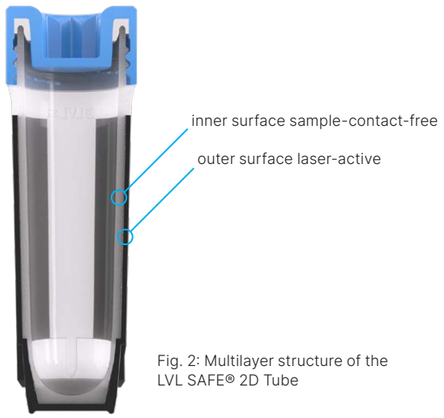


Fig. 2: Multilayer structure of the LVL SAFE® 2D Tube

In the second step, the molded sleeve is overmolded with a second plastic component that contains a small amount of color pigments and a specialized laser additive.

Targeted design and process engineering measures ensure that premature mixing of the two components is prevented at all times. The premolded sleeves and final molding stations are physically separated. In addition, each component is processed by its own dedicated injection unit, with fully independent hot runner systems that guide the respective polymer melts into the mold cavities.

This guarantees that neither color pigments nor laser additives can come into contact with the sample.

The closure component – the core of the tube assembly

The most technically demanding and simultaneously most sophisticated component of the tube assembly is the closure system.

For tubes with external threading, LVL employs the innovative two-component axial seal (TCAS) system.

The cap's base body, which also forms the thread geometry, is injection-molded in the first step using high-purity SAFE® L3 Medical Polypropylene. In a second step, a specially developed TPE (thermoplastic elastomer) is injected into this base body. The molecular chains of the two materials fuse permanently through thermoplastic bonding.

The excellent sealing performance is based on a clearly defined mechanism: the tube body applies a specified axial force onto the TPE seal, which compresses it in a controlled manner. This creates a reliable barrier against liquids and gases – the foundation of the axially acting sealing principle.

An additional advantage arises from the identical material used for the threaded components of both the tube and the cap. This ensures that both parts exhibit nearly identical thermal expansion and contraction behavior. As a result, the applied sealing forces are maintained even under extreme conditions, such as cryogenic temperatures.

Fig. 3: Closure components of the LVL SAFE® 2D Tubes with integrated TPE seal in axial seal design – ensuring reliable tightness without an O-ring

Advantages of the external thread

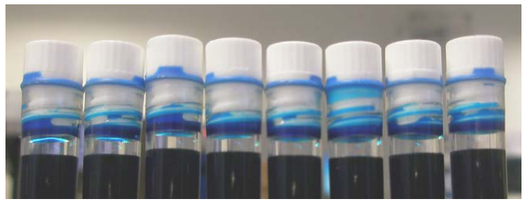
For many years, LVL has placed great emphasis on the use of external threading in the development of new sample containers. In addition to significantly improved storage efficiency, the lower risk of cross-contamination is a key factor. While the entire thread of internal-thread closures can become contaminated with the sample, only the small inner surface of the cap is exposed in external-thread containers.

Disadvantages of the internal thread

Once contaminated, internal-thread containers maintain constant contact between the sample fluid and the closure. Furthermore, the potential volume of sample carryover is significantly greater in internal-thread designs, presenting an elevated risk.

>>> For this reason, we advise against the use of tubes with **internal** threads.

Before centrifugation



After centrifugation

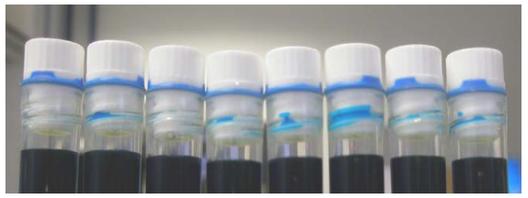


Fig. 4: High risk of carryover – unfavorable sample distribution – when using tubes with internal threading

Coding

Code uniqueness

The core principle of any automated or automation-ready sample storage system is the uniqueness of sample identification. Users who rely on a code that is unreadable to the human eye – rather than on a self-adhesive label or handwritten number – must be able to trust that this code is unique and will not be duplicated. We guarantee this. Every step in our process ensures that a previously assigned code cannot be accidentally reused. Most critical, however, is the final quality control: each rack is scanned, and every tube is cross-checked in real time against the database of all previously produced tubes. At this stage, any error would be immediately detected. Complete traceability of all produced codes – even years after delivery – is ensured.

Code stability

Code stability is at least as important as uniqueness. That is why LVL not only uses the most reliable technology – the fiber laser – but also applies a superior marking method. We do not laser onto foils or outer coatings, but directly onto the tubes or their laser-compatible components. Our technology does not merely alter the surface. Microscopic images of the base cross-section reveal that the laser penetrates approximately 50 microns into the surface – transforming the 2D code into a 3D code. This marking method represents the most reliable technical solution and offers the highest possible protection against mechanical, chemical, and thermal influences. Additionally, a protective rim was developed around the bottom of the tubes to shield against mechanical impacts. Friction or abrasion on the lab work surface no longer causes scratches – thus consistently ensuring high readability.

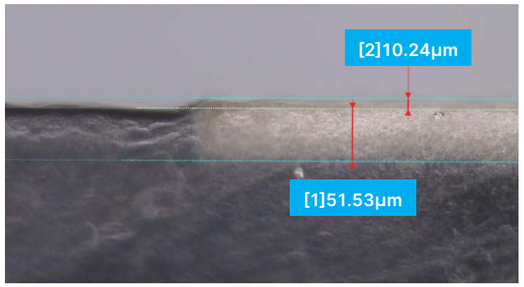


Fig. 5: Cross-section of the tube wall with laser-engraved 2D code, forming a durable 3D structure; image captured via optical microscopy (Keyence VHX-7000)

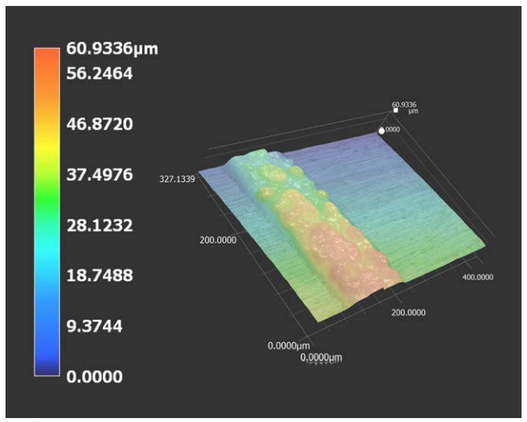
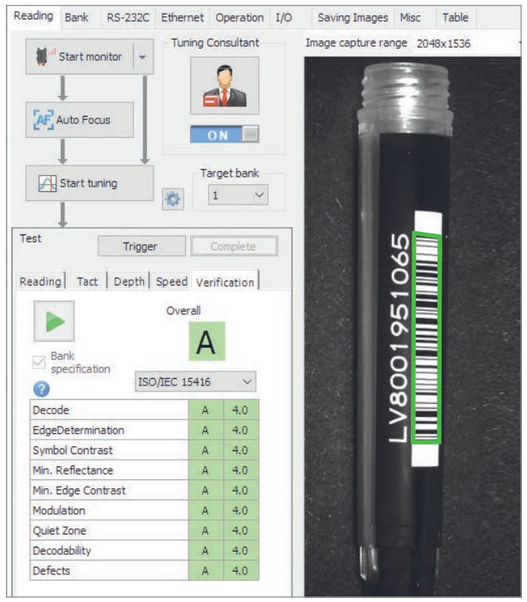


Fig. 6: Code surface measured on an LVL rack using a Keyence VHX7000

As shown in Figures 5 and 6, the laser-engraved code penetrates to a depth of over 50 microns. This depth ensures consistently stable code quality and guarantees reliable readability even after cryogenic storage and mechanical stress.

Code readability and numbering scheme

LVL SAFE® 2D Tubes are produced in compliance with ISO 15415. The quality level of the final 2D code is assessed using parameters such as contrast, modulation, and reflectance. Only tubes rated as Grade A or B are released for shipment, ensuring that even lower-grade scanners can reliably read the codes. And one seemingly small feature, with great impact: LVL is the only supplier to offer a 12-digit code as standard. The advantages of this become especially evident when using customer-specific numbering schemes with customized prefixes.



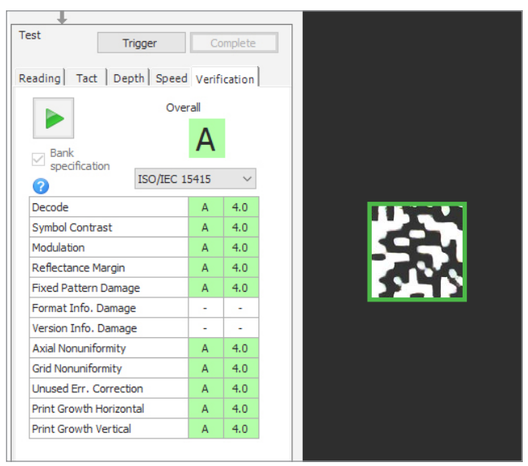


Fig. 7: 2D code verification report of an LVL SAFE® 2D Tube showing high contrast, error correction, and readability scores according to ISO 15415

Code redundancy

All LVL SAFE® 2D Tubes can be supplied with a 2D code on the tube bottom and redundant human-readable codes (on the bottom and side), and – depending on the type – with an additional 2D code or 1D barcode.

Fig. 8: Combination of four codes: 1D barcode and human-readable code on the side, 2D and human-readable codes on the tube bottom

Purity – sterility

Production sterility under clean room conditions

LVL SAFE® 2D Tubes are manufactured under controlled clean room conditions. Throughout the entire production process – including molding, assembly, and packaging – the environment is strictly monitored, for example by measuring particle concentrations according to ISO 14644 (ISO Class 8).

The products are classified as production sterile, meaning they are manufactured and packaged under low-bioburden conditions. This type of sterility is process-documented and validated, ensuring that the microbiological quality meets the intended use – especially for applications where terminal sterilization is not required or may be unsuitable due to material sensitivity.

Enhanced sterility for specialized applications

To ensure microbiological sterility of cryogenic tubes, LVL applies a standardized beta irradiation process. This method ensures effective inactivation of microorganisms through the targeted application of ionizing radiation. A defined radiation dose is administered under controlled conditions to destroy the DNA of bacteria, fungi, and other potential contaminants.

Compared to other sterilization methods such as autoclaving or ethylene oxide treatment, beta irradiation offers superior penetration depth and consistent effectiveness.

Sterilization is carried out in accordance with ISO 11137 and is fully documented. It applies to all product components that are irradiated in their final packaging. The applied radiation dose is validated and designed to achieve a sterility assurance level (SAL) of 10^{-6} – an industry standard also used for medical disposable products.

Summary of key quality features

LVL SAFE® 2D Tubes have been specifically developed to meet the highest demands in biobanking and automated sample processing. The foundation of their product quality is a high-purity, medically certified polypropylene (SAFE® L3) that is entirely free of additives and features a closed polymer matrix with minimal permeability. Both tubes and closures are precisely engineered to ensure mechanical stability, leak-tightness, and chemical inertness – even under extreme temperature conditions.

A key quality feature is the integrated two-component closure with TPE seal, which provides reliable sealing during repeated opening cycles and under cryogenic conditions. The laser-engraved 2D codes are permanent, tamper-resistant, and machine-readable; their high scanning reliability has been validated according to ISO standards. Manufacturing and packaging are carried out under cleanroom conditions, and tubes and racks are optionally sterilized to ensure the highest levels of purity and biocompatibility.

1 TESTING OF PHYSICAL PARAMETERS

For the long-term storage of samples under extreme conditions, the physical integrity of the storage containers is of critical importance. This chapter examines the leak-tightness of LVL SAFE® 2D Tubes against liquids and gases, as well as their mechanical stability under cryogenic temperatures, repeated handling, and centrifugation stress. The tests are based on established standards (including IATA and DIN 13279) and simulate realistic stress scenarios to demonstrate the tubes' suitability for safe and durable sample storage. Dead volume is also considered an important quality parameter, as it influences the efficiency of sample retrieval via pipetting.

1.1 Leak-tightness and torque

Gravimetric leak test (IATA test)

This test assesses the risk of environmental contamination from leaked sample material due to pressure drops during sample transport within a temperature range of −40°C to 55°C. It also helps determine the likelihood of contamination of the surroundings by leaked sample components. The testing conditions are based on § 173.27(c)(2i) and § 173.196(a)(7) of the United States Code of Federal Regulations (CFR), Title 49 – Transportation, for the evaluation of air transport safety of biological samples (IATA).

Method

The cryotubes and screw caps are pre-dried at 20°C under a pressure of 1 mbar and acclimatized under laboratory conditions. The tubes are filled with a colored antifreeze solution, sealed using a defined, tube-type-specific torque, and placed in a vacuum test chamber under a specified pressure-temperature profile. By applying negative pressure within the chamber, the test pressure is non-destructively created as a differential pressure across the tube wall and maintained throughout the duration of the test. A loss of seal integrity results in the leakage of test fluid. Using differential weighing, the resulting mass loss of the test substance can be quantified.

Tested samples: 2x LVL SAFE® Tube SX300 with matching screw caps.

(Analysis performed by: ILK Dresden)

Results

Figure 9 shows the distribution of the measured mass loss in graphical form for the LVL SAFE® 2D Tube.

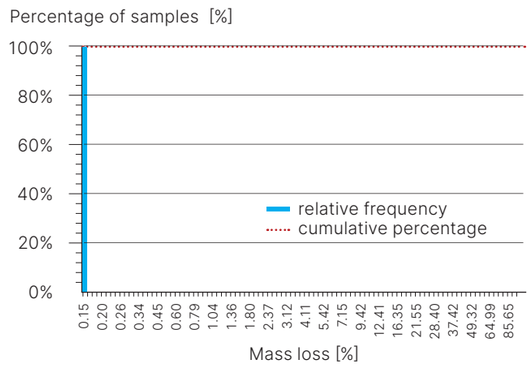


Fig. 9: Mass loss of LVL SAFE® 2D Tubes in the gravimetric leak test

All tube and screw cap combinations exhibited a mass loss of less than 0.1% and can be considered completely leak-tight.

CO_2 test

The leak-tightness of cryotubes against the ingress of carbon dioxide under dry ice transport conditions is evaluated. The test involves a defined incubation of the sealed cryotubes on dry ice for a period of 24 hours to simulate typical transport scenarios.

Method

After controlled filling of the tubes with a defined CO_2 absorption buffer, the samples are sealed with a torque of 7 Ncm, as specified by the manufacturer, and subsequently frozen. The frozen tubes are then incubated without pressure on dry ice at approximately −78°C for the duration of the simulated transport time. The sublimation of the dry ice creates a saturated CO_2 atmosphere.

After controlled thawing, the samples are brought to temperature equilibrium. Indirect detection of the absorbed carbon dioxide in the buffer is then carried out by measuring the buffer's pH value. Based on the measured pH, the absorbed CO_2 concentration is quantitatively determined, and its distribution is graphically represented and analyzed. The determined CO_2 concentration also allows for an assessment of the influence of the buffer systems used during sample storage. Tested samples: 2x LVL SAFE® Tube SX300 with matching screw caps.

(Analysis performed by: ILK Dresden)

Results

Table 1: Results of the CO_2-tightness test

The closures functioned flawlessly, showing no visible leakage and requiring no retightening, and successfully passed the test.

Test passed	Tight	Leaking	Median ($x_{50.0}$) [mg/ml]	Average [mg/ml]
Yes	96	0	<1 µg/ml	0.3

LVL SAFE® tubes can be used as inner packaging components in combination packaging in accordance with hazardous materials transport regulations. It is the user's responsibility to have the complete combination packaging certified in compliance with all applicable hazardous materials transport regulations issued by EASA, the U.S. Department of Transportation, ICAO, IATA, IMO, or other authorities.

Leak-tightness under cryogenic conditions from −196°C to −150°C – nitrogen permeation rate

Another highly relevant test scenario for evaluating leak-tightness is the potential permeation of nitrogen gas through the tube wall or leakage at the sealing interface at temperatures ranging from −196°C to −150°C. The aim of this test was to quantitatively determine the nitrogen concentration inside the tubes after storage in a saturated cold nitrogen gas atmosphere over varying periods of time.

Method

The tubes were sealed under a controlled CO_2 atmosphere (Corpadur C Endo, Westfalen) in an enclosed workspace (glove box) using a defined torque. The oxygen concentration inside the glove box was monitored with an SGM7.1 sensor (ZIROX, Greifswald) and maintained below 1% during filling. For each tube, the current oxygen level was recorded and subtracted from the gas-chromatographically determined value during analysis. After filling, the tubes were stored in a liquid nitrogen (LN_2) tank in cold nitrogen gas for 24 h, 72 h, and 168 h.

The gas composition inside the tubes was analyzed using a gas chromatograph (7890B, Agilent) equipped with a thermal conductivity detector. To perform the analysis, 200 µL of gas was drawn from a sealed port in the tube using a helium-filled syringe. After a 1:50 dilution, the injected gas sample was passed through a Carboxen column (Carboxen 1010, Supelco) at an oven temperature of 40°C. An increase in nitrogen concentration was used as an indicator of leakage. Data evaluation was based on peak area, using the composition of ambient air in the laboratory as the reference value.

(Analysis performed by: ILK Dresden)

Results

As shown in Figure 10, there was no measurable increase in the concentration of either gas during storage, within the margin of standard deviation. As expected – due to the negative exponential temperature dependence of the permeation coefficient – no permeation was detected for either gas. The nitrogen-to-oxygen ratio also remained stable within the measurement uncertainty. An increase in nitrogen concentration would indicate leakage at the closure. Since no such increase was detected, the tested tubes are considered leak-tight under cryogenic storage conditions.

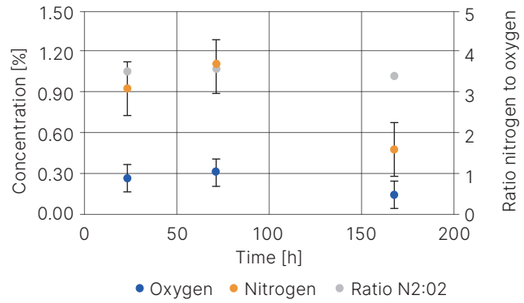


Fig. 10: Mean values of oxygen and nitrogen concentrations and the oxygen-to-nitrogen ratio after storage in a cold nitrogen tank

The measurement results clearly confirm the complete leak-tightness of the tube and closure system under realistic cryogenic storage conditions. No gas ingress through the vessel wall or the seal was detected – even after 168 hours. The tested tubes are therefore fully suitable for long-term storage in liquid nitrogen.

Leak-tightness of cap closures after repeated use

In routine biobank operations, it is common practice to open and reclose sample tubes multiple times – for aliquoting, analysis, or repackaging. This places particular demands on the mechanical durability and sealing performance of the cap closures. A key concern is whether repeated use may lead to material abrasion, which in turn could result in leaks. The objective of this test was to validate the leak-tightness of the tubes after up to 15 opening and closing cycles under realistic conditions.

Method

For the gravimetric leak-tightness test, 96 cryotubes of type LVL SAFE® LX1000 with matching screw caps were

used. The tubes were first subjected to 15 opening and closing cycles using an automated capper/decapper (SAFE® Cap 96 Channel DD) at a defined torque of 7 Ncm. They were then filled with a colored antifreeze solution, exposed to a defined pressure-temperature cycle in a vacuum test chamber (−40°C to +55°C, 99 kPa vacuum), and tested gravimetrically for mass loss due to leakage. A visual inspection for escaping liquid was also performed.

The potential loss of sealing material (seal integrity) after the specified number of cycles was also determined gravimetrically: the weights of the tube and screw cap were measured separately before sealing. After multiple opening and closing cycles, the weight of the screw cap and the sealed tube was measured again. The screw cap and outer part of the tube were cleaned separately with compressed air, while the tube was sealed with a silicone stopper to remove any loose particles, and reweighed. The tube was then filled with water, and any particles inside were flushed out and collected on filter paper for drying. The mass of the filter residue was determined by weighing the filter before and after rinsing with the water from the tube. Before weighing, all samples were conditioned for at least 4 hours to minimize potential effects from temperature or humidity fluctuations.

The difference between the initial weight before sealing and the weight after sealing, as well as differences before and after compressed air cleaning, were analyzed.

In addition, images of the sealing surface and the threads of both the tube and the screw cap were taken.

(Analysis performed by: ILK Dresden)

Results

The study demonstrated that all tested tubes remained fully leak-tight after 15 opening and closing cycles. Not a single tube exhibited visual leakage. The average mass loss was significantly below the allowable limit of 0.1% of the total weight.

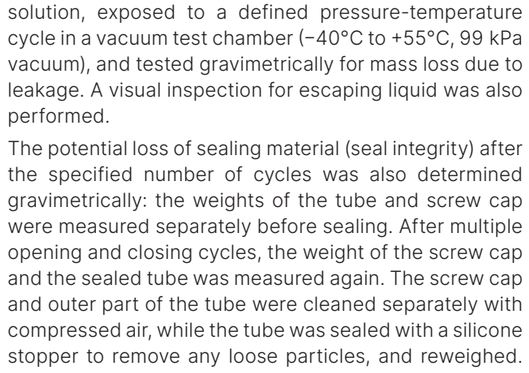


Fig. 11: Mass loss of LVL SAFE® 2D Tubes during 15 opening and closing cycles

Even under microscopic examination, no damage to the sealing surfaces was observed that could impair functionality.

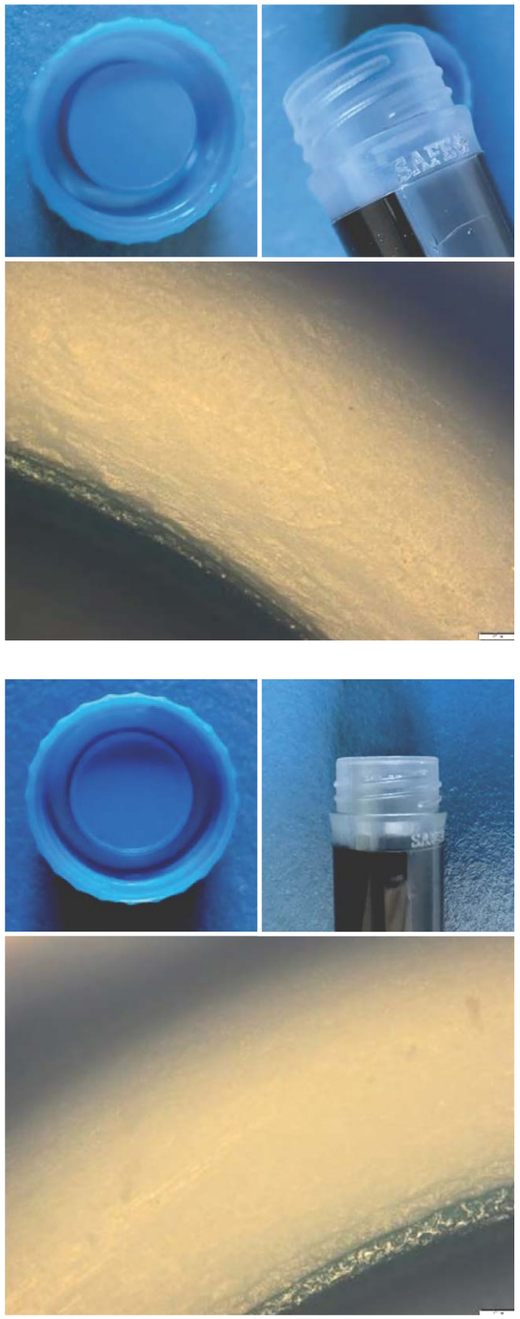


Fig. 12: Upper view: Cap (top left) and tube (top right) and cap seal at 50× magnification (bottom), after 5 opening/closing cycles

Lower view: Cap (top left) and tube (top right) and cap seal at 50× magnification (bottom), after 20 opening/closing cycles

Gravimetric analyses of filter residues after rinsing also revealed no relevant particle contamination.

The test results confirm that LVL SAFE® 2D Tubes remain reliably leak-tight even after 15 opening and closing cycles under laboratory conditions. The cap seal is mechanically robust and shows no significant wear that would compromise functionality. This ensures a high level of operational safety for applications requiring repeated access to samples.

1.2 Mechanical stability under centrifugation conditions

Centrifugation is an essential method for sample preparation in many laboratory processes. For the tubes, this represents a mechanical stress that may affect both seal integrity and material stability. Therefore, reliable suitability for defined centrifugation conditions is a key quality attribute.

Method

As part of quality assurance, LVL SAFE® 2D Tubes of types SX260, SX300, MX500, and LX1000 were specifically tested for their centrifugation resistance. Various rotor types were used under graduated conditions (Sigma 4-5KL with Rotor 11118 and Sigma 8KS with Rotors 11805/13845): 3,000 × g; 5,485 × g; 7,328 × g, each for 10 minutes at 4°C, Acc/Dec setting 9.

Centrifugation was performed with fully loaded racks. Samples were evenly distributed beforehand, properly sealed, and supported using suitable inserts.

(Analysis performed by: Sigma Laborzentrifugen, Osterode)

Results

All tested tubes showed no leakage, no visible material changes, and no functional impairments after centrifugation. The defined maximum specification for fully loaded racks is 7,328 × g; individual tubes can be used at up to 10,000 × g. The results were documented as part of the quality inspection process and confirm the suitability of the products for such applications.

LVL SAFE® 2D Tubes are fully suited for use under standardized centrifugation conditions. Structural integrity is maintained even at high accelerations – provided use remains within the specified limits. As such, the products meet key requirements for safe operation in both automated and manual laboratory workflows.

1.3 Summary and evaluation of physical parameters

The innovative product design, featuring a patented dual-seal system without O-rings, provides the foundation for the outstanding leak-tightness of LVL SAFE® 2D Tubes. Extensive testing – including the gravimetric leak test according to IATA, the CO_2 leak test, nitrogen permeation testing at −150°C to −196°C, and leak-tightness evaluation after repeated opening and closing – demonstrates consistently reliable sealing performance.

Even under mechanical stress, such as up to 15 closure cycles or centrifugation at up to 10,000 × g (for individual tubes), the tubes remain leak-tight and functionally stable.

The results confirm the unrestricted suitability of LVL SAFE® 2D Tubes for long-term cryogenic storage, secure sample transport, and use in diagnostics and automated processes.

2 TESTING OF AUTOMATION-RELATED QUALITY PARAMETERS

Modern tubes for biobanks and other sample repositories must be compatible with advanced robotic systems for liquid handling and automated storage. This requires precise dimensions and robust construction to ensure smooth and efficient operation.

2.1 Compatibility with robotics and storage systems

Even at relatively low levels of automation in the laboratory, the advantages of using 2D-coded tubes such as LVL SAFE® 2D Tubes are already apparent. With increasing automation, these advantages become even more significant and critical to efficiency and process reliability.

Whether for use in automated pipetting systems, storage in automated warehouse systems, or integration with other laboratory equipment, LVL SAFE® 2D Tubes and racks are designed from the ground up to seamlessly integrate into automated workflows. At the same time, integration into manual processes and adaptation to prevailing operational requirements – i.e., ease of handling – also play an important role in ensuring acceptance and user satisfaction.

Rack design

The LVL racks designed to hold LVL SAFE® 2D Tubes in SBS format were developed, engineered, and tested in collaboration with leading automation manufacturers.

This includes:

- consistent implementation and application of the SBS standard (ANSI SLAS 4-2004 (R2012), formerly known as ANSI/SBS 4-2004)
- defined specifications for the qualification and positioning of 1D, 2D, and human-readable (HR) codes
- optional placement of a 1D barcode on the east side of the racks (e.g., A12–H12 for SBS 96 format)
- 2D orientation code located on the bottom of the racks
- additional coding options available on the rack underside for automated sample processing (optional)
- patented rack design with guide pins and rounded well edges for error-free pick-and-place operations
- stable, collision-free anti-rotation pins for a reliable sealing process

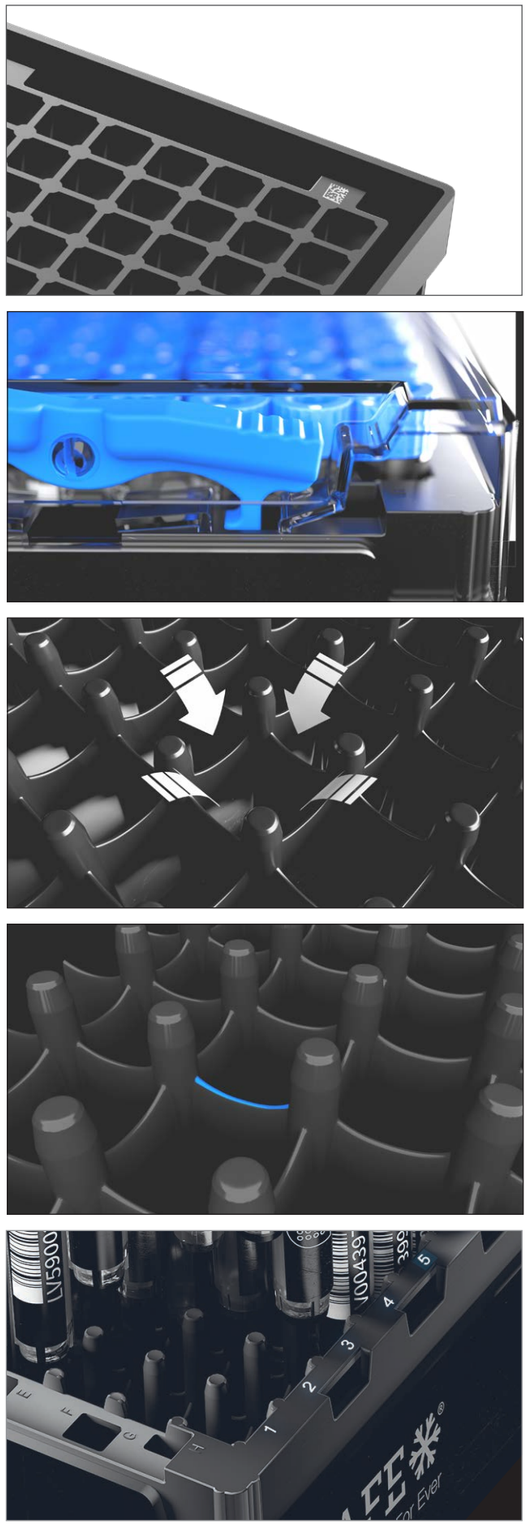


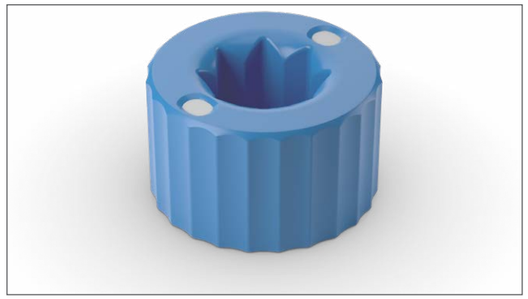
Fig. 15: Bottom view of LVL rack showing 2D orientation code and optional additional coding for robotic integration; patented LVL rack design for precise, automation-friendly sample handling

Tube and cap design

The design of the LVL SAFE® 2D Tubes is characterized by outstanding dimensional stability and precision, providing an excellent foundation for the reliable execution of pick-and-place processes.

Pick-and-place generally refers to the automated process in which products are picked up by a robot or machine and transported to a defined target position for placement.

Integrated, robustly constructed anti-rotation pins enable a secure, process-stable, and precisely repeatable sealing operation. In addition, the geometry of the closure components is specifically tailored to the requirements of automated systems – including capping/decapping units, robotic grippers, and extraction mechanisms.



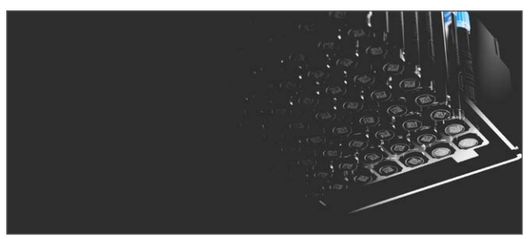


Fig. 16: Tube and cap design for reliable automation compatibility

Pick-and-place test in automated storage system

To evaluate the suitability of LVL SAFE® 2D Tubes for automated handling, a pick-and-place test was conducted under realistic conditions. The goal was to assess process stability and the mechanical durability of the tubes during automated storage logistics.

Method

The test was carried out using the TLM 864 Tube Laser Marker at room temperature (22°C). A total of 3,465 tubes (36 racks of 96 tubes each, type SX300) were processed. Each tube was automatically removed from a source rack and transferred to a pre-defined position in an empty target rack.

Results

During the 3,465 pick-and-place operations, only 3 errors occurred during tube placement, corresponding to an error rate of 0.086%. All errors were limited solely to the placement of tubes in the target rack. Despite these deviations, the automated process continued without interruption – the system completed the operation without disruption.

The very low error rate and uninterrupted continuation of the process confirm the high process reliability and automation suitability of LVL SAFE® 2D Tubes in conjunction with the TLM 864, even during large-scale batch operations under realistic storage conditions.

Capping/decapping

Although this tech note focuses on the SAFE® 2D Tube, Cap, and Rack system rather than devices and instruments, a brief note on LVL SAFE® capping technology is appropriate. This technology is based on two core principles: flexible cap driver design and precisely defined torque.

Because the technical properties of LVL SAFE® 2D Tubes – such as leak-tightness and automation compatibility – are dependent on the tube being properly closed, the question of how the tube is sealed is essential. Only with original LVL equipment – specifically designed to match the LVL tube design and its technical parameters – can the tube's full performance potential be reliably achieved and utilized in practice.

The flexible cap driver design, which incorporates tolerance-compensating metal balls, ensures that the cap is always gently gripped, held, and securely closed. The defined torque guarantees a perfectly sealed closure. This coordinated interaction between cap and device also prevents over-tightening of the threads.

Certified compatibility with leading storage and liquid handling systems

LVL SAFE® 2D Tubes have undergone extensive compatibility testing with automated storage systems from leading manufacturers. The tests were conducted in accordance with manufacturer-specific validation guidelines and included evaluations of handling, positioning accuracy, freeze behavior, and barcode readability. Based on these tests, official compatibility certificates were issued, confirming the full suitability of LVL Tubes for use in these systems. The certificates are available in the download section (see below).

2.2 2D code quality and code security

When 2D-coded tubes are used for sample storage, it must be ensured that samples can be identified not only more quickly but, above all, with complete reliability. The quality of both 1D and 2D codes should be objectively assessed according to international standards. Various criteria should be evaluated, including contrast, readability, error correction, alignment, and quiet zone, as these factors significantly affect reliable detection in automated processes. This evaluation serves as the basis for quality assurance and optimization of coding during production.

Method

The Keyence Code Verifier was used for the testing. The Keyence Code Verifier is a proven technology for standardized quality control of 1D and 2D codes. It is widely used in regulated industries such as pharmaceuticals, medical technology, and automotive, and ensures reliable, standards-compliant test results according to ISO/IEC 15415 and 15416.

The evaluation of 2D Data Matrix codes (DMS) was conducted in accordance with ISO 15415, while the assessment of 1D barcodes followed ISO 15416. From the individual measured parameters, an overall grade is calculated, ranging from A (excellent) to F (insufficient).

Ten test samples were evaluated per tube type. In addition to LVL SAFE® 2D Tubes, seven other commercially available tubes designed for similar purposes were tested and compared.

Results

The analysis provides a clear basis for comparing the coding performance of different tube types.

Table 3: Summary of 1D and 2D code quality evaluation for LVL SAFE® 2D Tubes and comparison products based on ISO/IEC 15415 and 15416 determined by Keyence Code Verifier – see also Figures 7

2D Data Matrix Code

LVL SAFE® 2D Tube/ Comparison tubes	LVL	1	2	3	4	5	6	7
Decode	A	A	A	A	A	A	A	A
Symbol contrast	A	A	A	A	A	A	C	B
Modulation	A	A	A	A	A	A	F	F
Reflectance margin	A	A	A	A	A	A	F	F
Fixed pattern damage	A	A	A	A	A	A	B	F
Axial nonuniformity	A	A	A	A	A	A	A	A
Grid nonuniformity	A	A	A	A	A	A	A	A
Unused err. correction	A	A	A	A	A	A	A	A
Print growth horizontal	A	A	B	B	A	B	A	F
Print growth vertical	A	B	D	A	B	A	B	F

1D barcode

LVL SAFE® 2D Tube/ Comparison tubes	LVL	1	2	3	4	5	6	7
Decode	A	F	A	N/A	N/A	A	A	A
Edge determination	A	F	A	N/A	N/A	A	A	A
Symbol contrast	A	A	A	N/A	N/A	A	B	A
Min. reflectance	A	A	A	N/A	N/A	A	A	A
Min. edge contrast	A	A	A	N/A	N/A	A	A	A
Modulation	A	F	A	N/A	N/A	B	D	D
Quite zone	A	F	A	N/A	N/A	A	A	A
Decodability	A	F	A	N/A	N/A	A	D	A
Defects	A	F	A	N/A	N/A	A	A	A

Grading scale for code quality
A = excellent to **F** = insufficient
N/A = Code not present

(Analysis performed by: LVL)

Based on the tabular summary (see Table 3), it is evident that the LVL SAFE® 2D Tubes meet all individual evaluation parameters – such as contrast, readability, error correction, alignment, and quiet zone – with the highest level of reliability. Notably, three of the comparison tubes, which are also marketed as suitable for automated sample storage, exhibited significant deficiencies that could lead to identification issues in practical use.

Only the LVL SAFE® 2D Tubes passed all individual tests for both code types with the highest reliability. They fully meet the requirements for secure identification and thus ensure dependable detection in automated processes.

2.3 Summary and evaluation of automation testing

LVL SAFE® 2D Tubes meet all requirements for use in highly automated laboratory liquid handling and storage systems. The high quality of the coding – validated according to ISO 15415 and 15416 – ensures error-free identification in robotic processes. The well-designed tube, cap, and rack system enables seamless pick-and-place handling and reliable integration into capping/decapping units, liquid handling systems and storage robots.

The practical test involving 3,465 transfers demonstrated an extremely low error rate of 0.086% with no process interruptions. Compatibility with leading storage systems is confirmed by official validation certificates.

This makes LVL Tubes exceptionally well suited for automated biobank workflows under cryogenic conditions.

3 TESTING FOR CHEMICAL INERTNESS

A key criterion in the development of SAFE® polypropylene for LVL was ensuring the chemical inertness of the container material. Chemical testing was therefore initiated to determine whether potentially migratable substances are released upon contact between the tube material and sample medium – thereby confirming the tubes' suitability for the safe storage of sensitive samples. The evaluation is based on established testing protocols from European Pharmacopoeia (Ph. Eur.) and United States Pharmacopeia (USP), as well as complementary analyses according to ISO standards.

3.1 Leachables and extractables

Originating from material testing and quality standards in the medical and pharmaceutical fields, the term "leachables and extractables" has become widely accepted for this type of evaluation. The DIN standard mentioned above also requires testing of biobank sample containers for critical classes of organic and inorganic substances.

Leachables are substances that can migrate from the container material into the sample under normal storage conditions, representing a potential risk during actual use.

Extractables are substances that can be forced out of the material under extreme test conditions. These tests are used to identify and evaluate potential leachables.

Broad screening of potential leachables and extractables using GC-MS and LC-MS

To comprehensively assess chemical safety, material extracts from the LVL SAFE® 2D Tubes underwent extensive analytical testing. Gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–mass spectrometry (LC-MS) were used to detect both volatile and non-volatile organic compounds with high sensitivity and specificity. To simulate realistic conditions, tests were conducted using an acidic buffer, a basic buffer, and isopropanol. The study was conducted in accordance with established regulatory guidelines and based on the requirements of ICH Q3D and ISO 10993-18.

Method

Sample preparation

For leachables (non-concentrated): One tube was filled with each test solvent (isopropanol, acidic buffer pH 4, basic buffer pH 9). Incubation was carried out for 72 hours at 37°C in a heating chamber. The samples were then subjected to HPLC-MS analysis without further processing.

For leachables (concentrated): Five tubes were filled with each of the test solvents (isopropanol, acidic buffer pH 4, basic buffer pH 9).

For extractables: Five tubes were mechanically shredded and extracted with isopropanol. These samples were also incubated for 72 hours at 37°C in a heating chamber. The contents were then combined and evaporated to dryness under a nitrogen stream at room temperature. The residue was reconstituted in 300 µL of various solvents, depending on the intended analytical method. Blank samples were also prepared for each solvent composition.

Chromatographic–mass spectrometric analysis

LC-MS: Single-quadrupole mass spectrometer with 1100 HPLC (Agilent, Waldbronn). Column: 125/2.1 Discovery C18 5 µm (Supelco); flow rate: 0.2 mL/min; column temperature: 45°C. Eluent: water/acetonitrile 5–95%.

GC-MS: 5890 GC with 5973N mass selective detector (Agilent, Waldbronn). Column: OPTIMA 5 HAT (30 m length, 0.25 mm ID). Injector temperature: 210°C. Column temperature program: 60°C to 320°C.

(Analysis performed by: University of Marburg, Department of Chemistry)

Results

Purity and quality of LVL SAFE® Tubes – leachables after 72-hour incubation with various media

The LC-MS analyses performed with isopropanol, acidic buffer, and basic buffer as incubation media revealed no relevant peaks for leachables in LVL SAFE® 2D Tubes compared to the blank sample. The chromatograms confirm the exceptionally high purity of the material and demonstrate that no potential contaminants are released (see Figure 17).

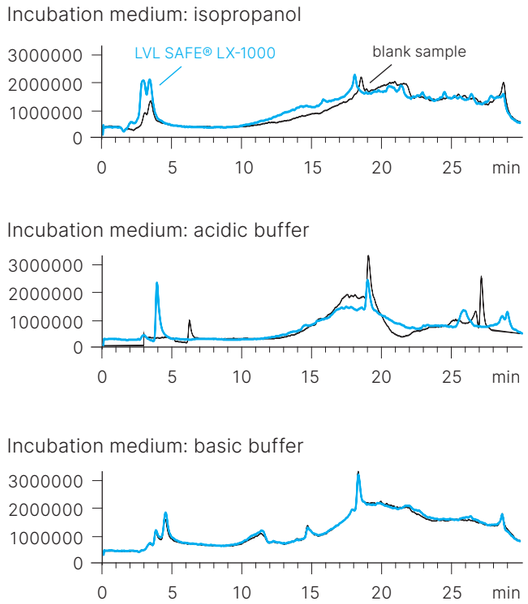


Fig. 17: LC-MS of non-concentrated samples
Legend: blue = LVL SAFE® LX-1000, black = blank sample

Additional analyses including comparison with market products

To increase variability in the testing conditions, the solutions were further concentrated to enhance the detection sensitivity. These tests included additional analyses using comparison products to assess the material quality of LVL SAFE® 2D Tubes in comparison.

The results (see Figures 18 and 19) clearly demonstrate the outstanding purity of the LVL SAFE Tubes. Even in the concentrated samples, no analytical peaks were observed in comparison to the blank that would indicate contamination or the release of undesirable organic substances from the tube material.

The quality of the LVL tubes is especially evident in the GC-MS analyses, which revealed a significant release of substances from the tested comparison tubes. Interpretation of the retention times and the corresponding analytical conditions suggests that these substances are hydrophobic in nature.

Comparison of the respective mass spectra with the NIST database indicates the presence of long-chain hydrocarbons.

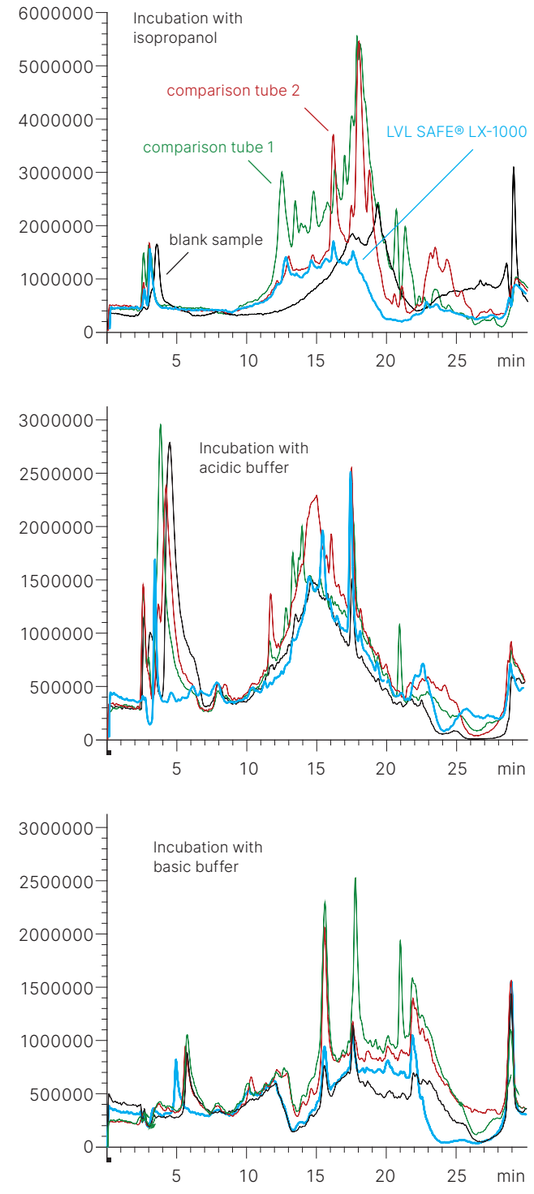


Fig. 18: LC-MS of concentrated samples for the detection of leachables, TIC ESI+

Legend: blue = LVL SAFE® LX-1000, black = blank sample, green = comparison tube 1, red = comparison tube 2

Analyses for the detection of targeted organic leachables and extractables were conducted using isopropanol as the incubation medium. Organic compounds are more likely to dissolve in isopropanol than in aqueous buffer solutions, making them more effectively detectable by GC-MS.

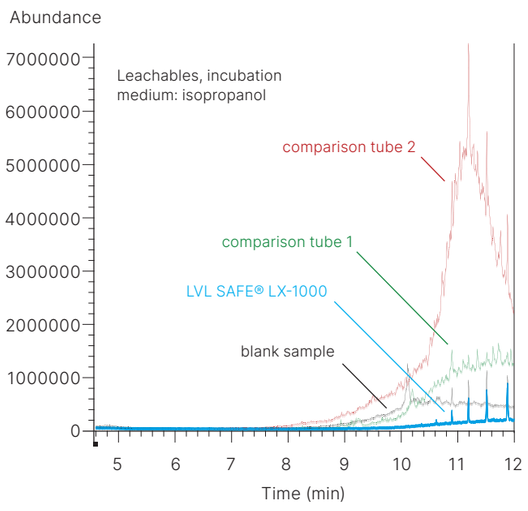


Fig. 19: GC-MS of concentrated samples, final temperature 320°C

Legend: blue = LVL SAFE®LX-1000, black = blank sample, green = comparison 1, red = reference sample 2

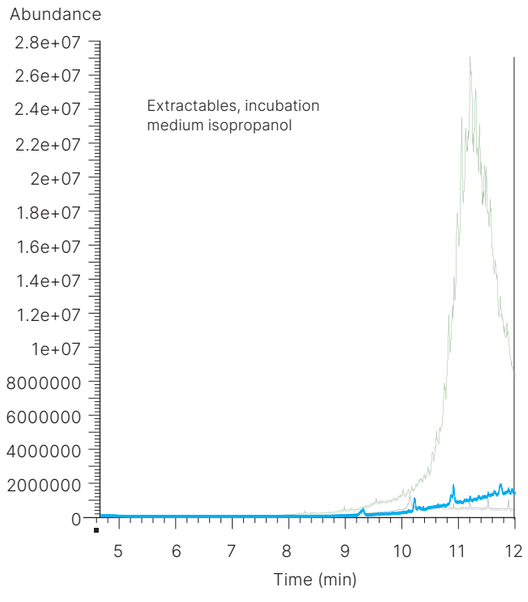


Fig. 19: GC-MS of concentrated samples, final temperature 320°C

Legend: blue = LVL SAFE®LX-1000, black = blank sample, green = comparison tube 1

The results clearly demonstrate the outstanding purity of the LVL SAFE Tubes. Even in the concentrated samples, no analytical peaks were observed in comparison to the blank that would indicate contamination or the release of undesirable organic substances from the tube material.

The quality of the LVL tubes is especially evident in the GC-MS analyses, which revealed a significant release of substances from the tested comparison tubes. Interpretation of the retention times and the corresponding analytical conditions suggests that these substances are hydrophobic in nature.

Comparison of the respective mass spectra with the NIST database indicates the presence of long-chain hydrocarbons.

Polypropylene-specific contaminants

During the production of PP tubes, residual substances from the manufacturing process – such as mold release agents, additives, or other chemical compounds – can remain in the material. To ensure purity beyond general leachables and extractables analysis, this study examined four substances typically found in plastics. The aim was to compare LVL SAFE® 2D Tubes with two anonymized comparison tubes (referred to as comparison tubes 1 and 2) and to identify any differences in material purity.

- **1-dodecanol is used in polypropylene processing as a lubricant or mold release agent.**
- **Pentadecane can be formed as a thermal degradation product of additives or oligomers during the processing or storage of polypropylene.**
- **3,4-dimethylbenzaldehyde is a known degradation product of dibenzylidene sorbitol-based clarifying agents, which are commonly used in polypropylene.**
- **Ethyl-4-ethoxybenzoate is used as an external donor in Ziegler–Natta catalyst systems during polypropylene synthesis and may remain as a residue in the material.**

Method

The analysis was performed in accordance with EN ISO 10993-12 “Biological evaluation of medical devices – Sample preparation and reference materials.”

Three to five tubes (including caps and sealing components) were fully shredded and incubated in a defined mixture of four different solvents:

- 80% ethanol/20% water
- 80% 2-propanol/20% water
- 100% water
- 100% dimethyl sulfoxide (DMSO)

Extraction was carried out at 37 ± 1°C for 72 hours. The extracts were then analyzed by GC-MS for the four target substances. Solvent evaporation was intentionally omitted due to the differing properties of the extraction media. Results were compared with blank and negative controls to ensure that no contamination occurred from the procedure itself.

(Analysis performed by: ILK Dresden)

Results

LVL SAFE® 2D Tubes showed no detectable levels of any of the target substances. All concentrations were below the detection limits:

- <0.5 mg/L for 1-dodecanol
- <0.1 mg/L for 3,4-dimethylbenzaldehyde and pentadecane
- <0.2 mg/L for ethyl-4-ethoxybenzoate

In contrast, substances were detected in relevant concentrations in comparison tubes 1 and 2 – specifically, elevated levels of 1-dodecanol in comparison tube 1 and ethyl-4-ethoxybenzoate in comparison tube 2 following extraction with 2-propanol/water. These results indicate the presence of residues that could potentially compromise the integrity of biological or chemical samples.

Table 4: Detected concentrations of the four target substances in blank, negative control, and extract samples

Sample	Extraction medium	1-dodecanol mg/L	3,4-dimethylbenzaldehyde mg/L	Pentadecane mg/L	Ethyl-4-ethoxybenzoate mg/L
Blank sample	Dimethyl sulfoxide	<0.5	<0.1	<0.1	<0.2
Blank sample	80% ethanol/20% water	<0.5	<0.1	<0.1	<0.2
Blank sample	80% 2-propanol/20% water	<0.5	<0.1	<0.1	<0.2
Blank sample	Water	<0.5	<0.1	<0.1	<0.2
LVL SAFE® Tube	Dimethyl sulfoxide	<0.5	<0.1	<0.1	<0.2
LVL SAFE® Tube	80% ethanol/20% water	<0.5	<0.1	<0.1	<0.2
LVL SAFE® Tube	80% 2-propanol/20% water	<0.5	<0.1	<0.1	<0.2
LVL SAFE® Tube	Water	<0.5	<0.1	<0.1	<0.2
Comparison tube 1	Dimethyl sulfoxide	<0.5	<0.1	<0.1	<0.2
Comparison tube 1	80% ethanol/20% water	<0.5	<0.1	<0.1	<0.2
Comparison tube 1	80% 2-propanol/20% water	2.8	<0.1	<0.1	<0.2
Comparison tube 1	Water	<0.5	<0.1	<0.1	<0.2
Comparison tube 2	Dimethyl sulfoxide	<0.5	<0.1	<0.1	<0.2
Comparison tube 2	80% ethanol/20% water	<0.5	<0.1	<0.1	<0.2
Comparison tube 2	80% 2-propanol/20% water	<0.5	<0.1	<0.1	1.1
Comparison tube 2	Water	<0.5	<0.1	<0.1	<0.2

Another noteworthy finding was the visible degradation of the sealing material in comparison tube 2 after extraction with DMSO. While this had no effect on the concentration of the analyzed substances, it does indicate potential weaknesses in material stability.

None of the target substances were detected above the limit of detection in the LVL SAFE® 2D Tubes. In contrast, one of the substances was detected in each of the comparison tubes, depending on the extraction medium.

3.2 Detection of the absence of heavy metal residues

Heavy metals can also leach from polypropylene sample containers under inadequate material or manufacturing conditions, potentially compromising the quality and integrity of the samples. Accordingly, the DIN standard referenced above rightly requires proof that the materials used are free from heavy metals.

Method

The analysis was conducted in accordance with DIN EN ISO 10993-18 (2021), "Biological evaluation of medical devices – Part 18: Chemical characterization of materials used in medical devices as part of a risk management process."

Extraction conditions: Single extraction / 72 hours / 37°C / stirring at 30 rpm in water. Eleven test samples, each with 1 mL, and a total surface area of 12 cm^2 per sample.

After extraction, the samples were removed from the extraction vessels, dried, and inspected for any changes in color, size, or other properties, which were recorded when observed. Photographic documentation was then created. The test items were subsequently repackaged in their original containers, marked as extracted, and grouped per project.

ICP-MS –quantitative analysis

The ICP-MS fingerprint analysis was performed using a Perkin Elmer NexION 300X instrument. Element content was determined based on DIN EN ISO 17294-2. A five-point calibration was used for each element. The actual elemental concentration was calculated considering the exact extraction volume, the number of samples per extraction, and/or other relevant project-specific parameters provided by the client.

The limits of quantification (LOQ) were: 0.0001 µg/mL for nickel, arsenic, cadmium, mercury, and lead; 0.001 µg/mL for chromium. (Analysis performed by: Clean Controlling Medical, Emmingen-Liptingen)

Results

The test samples showed no signs of change after extraction. No alterations in color or dimensions were observed. The extract was clear and colorless, with no particles, precipitates, or flocculation detected.

As shown in Table 5, no heavy metal elements were detectable within the achievable limits of quantification.

Table 5: Results of heavy metal analysis in LVL SAFE® 2D Tubes

Element	Element concentration	
	[µg/mL]	[µg/item]
Arsenic	<LOQ	<LOQ
Cadmium	<LOQ	<LOQ
Chromium	<LOQ	<LOQ
Lead	<LOQ	<LOQ
Mercury	<LOQ	<LOQ
Nickel	<LOQ	<LOQ

The analyses confirm that the material used is free from detectable concentrations of heavy metals and fully complies with current requirements for material purity in the storage of sensitive samples.

3.3 Summary and evaluation of chemical inertness

The comprehensive analysis of the chemical inertness of LVL SAFE® 2D Tubes clearly demonstrates their exceptional material quality and production purity. In all tests conducted – from broad screening for potential leachables and extractables to targeted analysis of common plastic-related contaminants – the LVL tubes not only met regulatory standards but consistently outperformed comparison products.

In particular, the analytically undetectable concentrations of migratable substances highlight the high chemical stability of the SAFE® L3 Medical Polypropylene used. Moreover, the low or non-existent signal intensities in the LC-MS and GC-MS analyses confirm that neither polymer residues nor additives – such as mold release agents or lubricants – compromise the integrity of sensitive samples. This stands in contrast to the comparison products, some of which exhibited significant contamination.

These results can be clearly attributed to two key factors: the deliberate selection of a high-purity, medically classified polymer material and a strictly controlled, additive-free production process. The intentional avoidance of process-related additives, combined with a multi-stage injection molding process free from material mixing, ensures a homogeneous polymer structure with minimal interaction with the sample – an essential requirement for chemically inert storage conditions.

Taken together, the test results confirm the exceptional suitability of LVL SAFE® 2D Tubes for the safe and stable long-term storage of chemically and biologically sensitive samples. They not only meet but exceed the requirements of relevant standards and set new benchmarks in terms of material purity and manufacturing quality.

4 LOW BINDING – TESTING FOR INTERACTION WITH BIOLOGICAL SUBSTANCES

The reliable storage of proteins and nucleic acids at very low concentrations places special demands on the sample container material. Potential adsorption of these substances to the vessel walls can impair sample recovery and distort analytical results. SAFE® 3L polypropylene is specifically designed to minimize the undesired binding of biological molecules. This chapter systematically evaluates the low binding properties of the material for proteins and DNA to ensure sample integrity during long-term storage and analysis.

4.1 Low binding – protein

For this comparison, a protein mixture (see Table 1) was selected to include proteins with varying degrees of hydrophobicity and abundance. This composition reflects the physicochemical properties of a typical laboratory protein sample and is particularly well-suited for evaluating the general applicability of the material across diverse use cases.

Method

The protein mixture (see Table 6) was stored in LVL SAFE® LX1000 tubes and in various comparison tubes from other manufacturers – each with declared low binding properties – for both 1 hour at room temperature and 4 weeks at –20°C, with all conditions tested in triplicate. For analytical processing, the samples were digested with trypsin using the S-Trap method and analyzed by mass spectrometry (MS). To normalize for technical variation, a peptide standard (iRT, Biognosys) was added to each sample.

Table 6: Composition of the protein mixture used, including selected proteins, molecular size, and abundance in the sample

Protein	Accession no.	Chain length	MW (Da)	nmol protein / sample
α-Lactalbumin	P00711	122 aa	14000	10
Glyceraldehyde-3-phosphate dehydrogenase	P46406	334 aa	37000	10
Alcohol dehyrogenase	P00330	348 aa	36800	1
Soybean trypsin inhibitor	P01071	181 aa	20000	1
Lysozyme	P00698	129 aa	14300	1
Bovine serum albumin	P02769	582 aa	69000	0.1
Carbonic anhydrase	P00921	261 aa	29000	0.1

In the bar charts, protein losses are presented as the difference in averaged peak areas of the extracted ion chromatograms between the 1-hour and 4-week storage conditions.

LVL SAFE® LX1000 tubes and six additional low-binding comparison products were included in the study.

(Analysis performed by: University of Greifswald, Proteomics Department)

Results

As shown in Figure 21, the LVL SAFE® 2D Tubes exhibited the lowest protein loss after four weeks of storage at –20°C compared to all other tested products. Although some degree of substance loss was observed across all tube types, the results confirm the above-average suitability of LVL SAFE® 2D Tubes for protein-based applications.

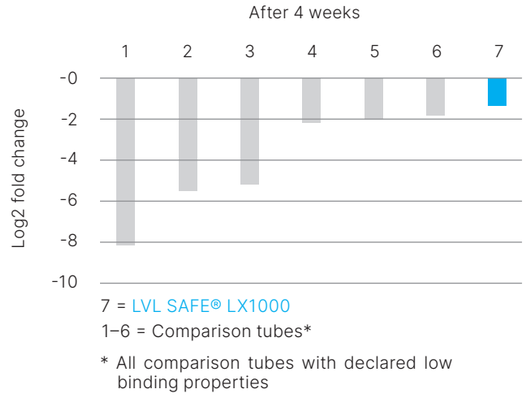


Fig. 21: Protein recovery after 4 weeks of storage at –20°C in LVL SAFE® 2D Tubes and six comparison tubes labeled as “low-binding”.

Three other tube types showed losses of a comparable magnitude and can therefore also be considered generally suitable. Surprisingly, the results for comparison products 1, 2, and 3 – despite being labeled as “low protein binding” – showed significantly higher losses.

Since the method used does not allow for differentiation between adsorption and potential protein instability, the focus of this evaluation is intentionally placed on the relative performance comparison between the tested systems. The results clearly demonstrate that LVL SAFE® 2D Tubes stand out as a particularly high-performing option in the competitive landscape.

4.2 Low DNA binding – preservation of sample quality and quantity

Method 1

Genomic DNA (gDNA) and cell-free DNA (cfDNA) were isolated from human blood samples and analyzed using established methods to evaluate the preservation of DNA quantity and quality after 1, 7, and 118 days of storage in LVL SAFE® SX300 tubes at –80°C and above liquid nitrogen. DNA was extracted from whole blood using the NucleoSpin Blood L Kit (Macherey & Nagel).

cfDNA was extracted from plasma using the Mag-Bind® cfDNA Kit. Equal sample volumes were distributed across the different tube types and storage conditions in triplicates. An initial measurement (d0) was performed on the aliquoted samples using the Qubit dsDNA Broad Range Kit (Life Technologies GmbH) and the Fragment Analyzer Genomic DNA 50kb Kit (Agilent Technologies). Measurements were repeated after 7 days under the specified storage conditions using the same tubes.

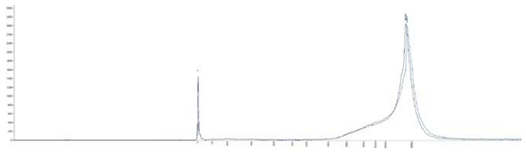
(Analysis performed by: University Hospital Carl Gustav Carus Dresden / BioBank Dresden)

Results

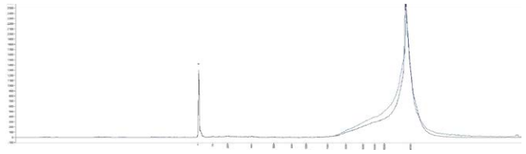
Preservation of nucleic acid fragment lengths during storage

In the electropherogram, peaks are shown relative to the size and concentration of the DNA fragments. The figures below demonstrate the high consistency in the fragment profiles of both gDNA and cfDNA – indicating structural preservation – during storage at –80°C and in the vapor phase above liquid nitrogen.

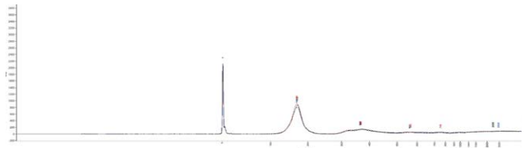
gDNA – 7 days of storage at -80°C
Day 0 = black, day 7 = blue



gDNA – 7 days of storage above liquid nitrogen
Day 0 = black, day 7 = blue



cfDNA – 118 days of storage at -80°C
Day 0 = black, day 7 = blue, day 118 = red



cfDNA – 7 days of storage above liquid nitrogen
Day 0 = black, day 7 = blue

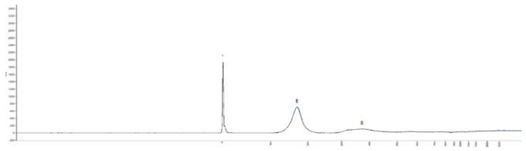


Fig. 22: Overlays of electropherograms for different types of DNA under different storage conditions

Low DNA binding

Unwanted binding of DNA to the inner surfaces of the tubes would result in a reduction of DNA concentration within the sample. Figure 23 shows the comparison of cfDNA concentrations between the initial storage day and after 1 and 7 days in various tubes, including LVL SAFE® 2D Tubes and several comparison tubes.

Due to the combination of the analytical method’s inherent variability – specified by the manufacturer as ±15% – and the limited sample volumes that could reasonably be obtained from patient specimens, the graphed results should be interpreted as trend data rather than absolute values.

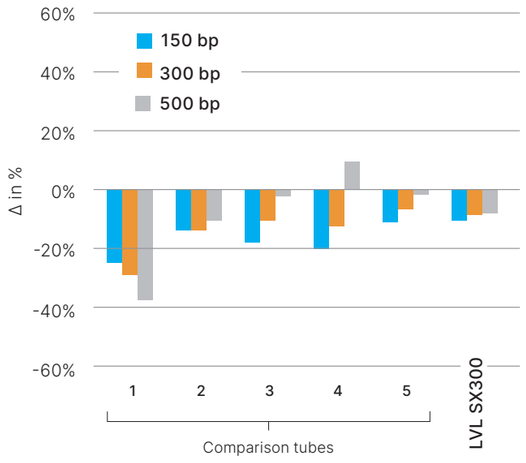


Fig. 23: Comparison of cfDNA concentrations in various tubes after 1 day of storage at −80 °C
(Method: Fragment Analyzer)

In Figure 23, clear differences can be seen in the measured concentrations of cfDNA after 1 day of storage. Notably, comparison tube 1 shows a significantly greater loss of various fragment concentrations. The lowest losses were observed in the LVL SAFE® tube and comparison tube 5. When interpreting the results, the measurement uncertainty of ±15% must be taken into account – especially with regard to changes in the positive range, given that the electropherograms above show no shift in fragment lengths.

Method 2

As part of an external comparison test, reagent tubes from five different manufacturers were filled with a defined amount of human L1 DNA. The samples were incubated for 24 hours at room temperature. The remaining DNA concentration was then quantified using real-time PCR (SOP 99880). The measurements were recorded as Ct values (cycle threshold), where lower Ct values indicate higher DNA concentrations.

Three concentration levels were evaluated: 20, 60, and 200 pg/µL.

(Analysis performed by: LADR Bremen)

Results

The results in Table 7 show that LVL SAFE® 2D Tubes exhibit the lowest Ct values across all concentration levels – indicating superior preservation of DNA quantity and integrity.

Table 7: Comparison of Ct values for DNA stability (after 24 h at room temperature)
Real-time PCR results at 20, 60, and 200 pg/µL L1 DNA

Tube type	20 pg/µL	60 pg/µL	200 pg/µL
LVL SAFE® 2D Tube	17.3	15.2	13.2
Comparison tube 1	18.9	15.8	13.3
Comparison tube 2	18.0	15.2	13.1

In direct comparison, both comparison products showed higher Ct values – particularly at the lowest concentration of 20 pg/µL – indicating a reduced DNA yield. At higher concentrations, the results converge, though LVL SAFE® 2D Tubes consistently demonstrate equal or superior stability.

These findings confirm the high suitability of LVL SAFE® 2D Tubes for storing sensitive DNA samples, especially at low concentrations typical of molecular biology or forensic applications.

4.3 Summary and evaluation of binding behavior

LVL SAFE® 2D Tubes exhibit excellent low binding properties for both proteins and DNA. Independent studies showed significantly lower losses of proteins and nucleic acids after extended storage compared to selected comparison products – including tubes specifically marketed as low-binding. Sample integrity was preserved in all tests.

Both mass spectrometry analyses for proteins and fragment analyses, as well as qPCR-based stability tests for DNA, confirmed the high material quality and minimal surface binding. This makes LVL SAFE® 2D Tubes ideally suited for applications involving sensitive biomolecules, where maximum recovery and reliable quantification are essential.

5 TESTING FOR BIOCOMPATIBILITY AND ABSENCE OF CONTAMINATION

Proof of the absence of biological contamination is another essential standard and quality criterion, as cryopreservation is widely used for biological samples, and contamination could significantly compromise sample purity and quality.

5.1 Absence of contamination

Human DNA, DNase, RNase

Method

Sample extraction

Twelve tubes (three from each rack) were rinsed with DNA- and nuclease-free bidistilled water.

Test methods for human DNA

Polymerase chain reaction (real-time PCR) in accordance with SOP 99880 / 1.0; for DNase: enzymatic DNA digestion according to SOP 100570 / 1.0; for RNase: enzymatic RNA digestion according to SOP 100571 / 1.0

(Analysis performed by: LADR Bremen)

Results

Human DNA: not detected (<0.5 pg/µl)

DNase: no activity detected (<10^{-6} Kunitz units)

RNase: no activity detected (<10^{-9} Kunitz units)

Endotoxin (Ph. Eur. 2.06.14.): <0.001 EU/ml
(kinetic turbono-dimetric, 37°C, 60 min.)

The sample containers showed no detectable DNA or RNA contamination. They fully comply with DIN requirements in this regard and are suitable without restriction for use in the relevant application areas.

Mycoplasma

Method

The test material was examined for the presence of mycoplasma using real-time PCR (RT-PCR) in accordance with Ph. Eur. 2.6.7 (11th Edition, 2023). The method was validated according to Ph. Eur. 2.6.7 and 2.6.21. Sample preparation followed protocol 4-09-SOP-01-068. DNA was isolated using the Venor®GeM Sample Preparation Kit (Minerva Biolabs GmbH), and RT-PCR was performed using the Venor®GeM qEP Kit (Minerva Biolabs GmbH). A total of 10 µL of DNA solution was used per RT-PCR reaction. The detection limit was 10 CFU/mL.

(Analysis performed by: BIOSERV Analytik und Medizinprodukte GmbH / HygCen Schwerin)

Results

No mycoplasma DNA was detected in the test material. The material is confirmed to be free from mycoplasma contamination.

Pyrogens, endotoxins

Method

The testing was conducted according to EN ISO 10993-11:2018 using the monocyte activation test (test report PB2024-0292_SN 38194 from 2024-03-01) and EN ISO 10993-11:2018 using the LAL-test.

(Analysis performed by: HygCen Schwerin)

Results

The monocyte activation test for pyrogens conducted in accordance with EN ISO 10993-11:2018 detected no pyrogens within the test sample.

Similarly, the LAL test for endotoxins conducted in accordance with EN ISO 10993-11:2018 detected no endotoxins within the test sample.

BSE/TSE safety – absence of animal-derived materials

Only high-purity polypropylene is used in the production of LVL SAFE® 2D Tubes. According to the manufacturer, this material is entirely free from additives of animal or human origin. It therefore fully complies with the requirements of the “Note for Guidance EMA/410/01 (rev. 3)” regarding safety in relation to BSE and TSE transmission. The material certification further confirms that no genetically modified organisms (GMOs) are used in production.

As a result, the polypropylene used is not only free from animal-derived substances but also meets ethical and regulatory standards, making it especially safe and versatile – even for sensitive applications in medical and life science research.

5.2 Cytotoxicity according to ISO 10993

Proof of cell compatibility is essential for use of materials in sample storage, especially in medical and diagnostic applications. Cytotoxicity testing is conducted to rule out any potentially cell-damaging effects caused by the material itself or by any additives it may contain. This evaluation is a required component of the comprehensive biological assessment in accordance with ISO 10993-5 and is also mandated by USP <87>.

Method

The polypropylene material used (LVL SAFE® LL Medical) underwent in vitro cytotoxicity testing in accordance with ISO 10993 (2013 edition) and USP 36/39 Chapter <87>. The goal was to determine whether material extracts exhibit toxic effects on cultured cells. L929 mouse fibroblast cell lines were typically used for the test; the evaluation criteria included morphological changes, cell lysis, and growth inhibition.

(Analysis performed by: polypropylene manufacturer)

Results

The cytotoxicity test revealed no indications of harmful effects on cells. The material was classified as noncytotoxic and fully meets the requirements of ISO 10993-5 and USP <87>. No relevant cytotoxic responses were observed, and the material extracts did not affect cell growth or morphology.

The tested polymer components of the LVL SAFE® 2D Tubes exhibit excellent cell compatibility and are considered biologically safe according to current standards. This makes them well suited for use in contact with biological tissues or cells, as well as in sensitive molecular biology applications.

5.3 Proof of sterility

Sterility of the packaging system is essential for the use of cryotubes in medical research and biobanking practice. To prevent microbial contamination and ensure the integrity of biological samples, certified sterility in accordance with pharmacopeial standards is required. In the present test, the microbiological sterility of a production batch was verified according to the standards of the European Pharmacopoeia (Ph. Eur. 2.6.1) and the United States Pharmacopeia (USP <71>).

Method

The sterility test was performed under GMP conditions following Ph. Eur. 2.6.1 (11.6) and USP-NF <71> (Issue 3, 2024). One production unit of the LVL SAFE® 2D MX500 product was tested.

Twenty representative samples were taken from the packaging unit. From each sample, 10 tubes were introduced directly – without rinsing or filtration – into the test media (direct inoculation method). Two different nutrient broths were used: CASO broth (soybean casein digest broth) for detection of aerobic and facultatively anaerobic microorganisms; thioglycollate broth for detection of anaerobic microorganisms.

Each medium was used in a volume of 400 mL. Samples were aseptically inoculated under clean room conditions. Incubation was carried out for a minimum of 14 days under controlled temperatures according to the requirements of the respective media (typically 20–25°C for CASO, 30–35°C for thio).

To ensure the quality of the nutrient media, growth controls were also performed using reference strains: Bacillus spizizenii (aerobic, for CASO broth) and Clostridium sporogenes (anaerobic, for thio broth).

(Analysis performed by: Labo LS Bad Bocklet)

Results

No macroscopically visible growth was observed in any of the test samples throughout the incubation period.

Test method: Direct inoculation

Total number of samples per medium: 10

Incubation period: Minimum 14 days

Overall assessment: No macroscopically visible growth

Both the direct assessment of the test media and the positive controls confirmed valid and flawless results. The test organisms grew as expected, validating the test conditions.

The tested tubes and packaging units fully complied with pharmacopeial sterility requirements. This confirms their suitability for sterile applications in sample archiving and molecular diagnostics. For users, this ensures the highest level of microbiological safety in the handling and storage of sensitive biological materials.

5.4 Summary and evaluation of biocompatibility and purity

LVL SAFE® 2D Tubes meet the highest standards for biological purity and biocompatibility. Certified laboratories confirmed the absence of relevant contaminants such as DNase, RNase, human DNA, endotoxins, pyrogens, mycoplasma, and BSE/TSE-related risks. In addition, noncytotoxicity was verified in accordance with ISO standards.

Product sterility is ensured both through a process-based clean room manufacturing environment and through terminal beta irradiation, validated according to ISO 11137. As a result, the tubes are fully suited for use in sensitive biological applications and regulated environments.

6 SUSTAINABILITY AND EFFICIENCY

Efficient use of storage capacity and reduction of energy consumption are critical factors in biobanking. With the LVL SAFE® 2D Tubes and corresponding SBS racks, LVL technologies offers a space- and energy-optimized solution that also minimizes environmental impact.

Method

The analysis of space and energy savings was conducted through a direct comparison between standard cryoboxes (9 × 9 format) and LVL SAFE® SBS high-density (HD) racks. Typical capacities, energy consumption, and investment costs for freezers were taken into account. The data was based on:

- the maximum number of tubes per rack and freezer
- specific energy consumption (kWh) per freezer
- the CO_2 intensity of the EU electricity mix (226 g CO_2/kWh), and
- the average electricity price in the EU (30 cents/kWh)

Practical scenarios examined tube volumes of 300 µL, 500 µL, and 1000 µL stored in LVL HD138 SBS racks. Freezer space requirements were also evaluated to quantify savings in storage capacity and energy consumption. (Analysis performed by: LVL)

6.1 Space savings – maximum storage in minimum space

The optimized design of the LVL SAFE® SBS high-density (HD) racks allows significant space savings. Compared to conventional cryoboxes, LVL HD138 racks can store up to 80% more samples within the same footprint. For example, storing 238,464 samples in LVL HD138 SBS racks with SX300 2D tubes requires only one freezer instead of five, reducing the space requirement from 5 m² to just 1 m².

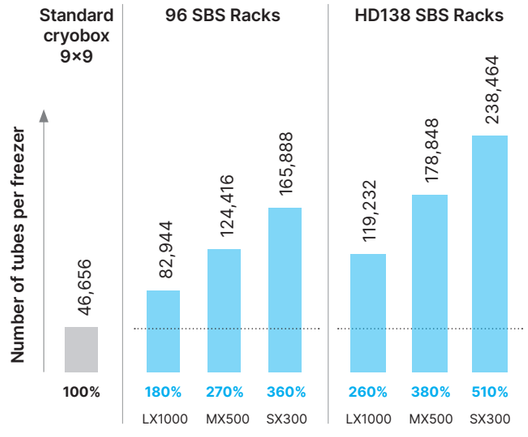


Fig. 24: Space savings with LVL HD138 SBS racks compared to standard cryoboxes

6.2 Energy efficiency – reducing consumption and emissions

The higher packing density of LVL SAFE® SBS racks not only reduces required space but also significantly lowers energy consumption. By reducing the number of freezers needed, annual energy use for 100,000 samples can be reduced by up to 6,922 kWh, which corresponds to a reduction of 1,564 kg of CO_2 emissions per year.

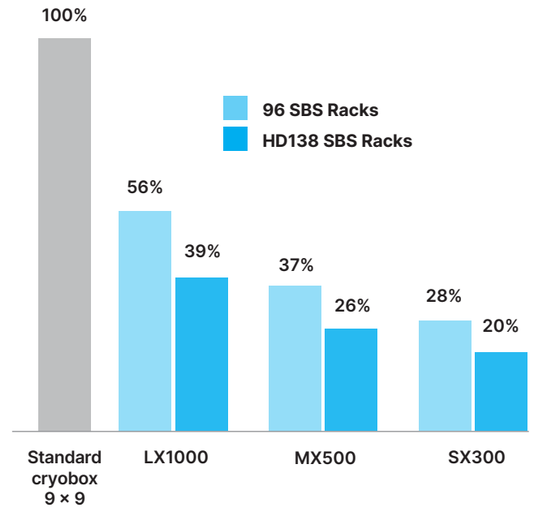


Fig. 25: Reduction in energy consumption and CO_2 emissions using LVL SBS racks

6.3 Summary and evaluation of sustainability aspects

The use of smaller sample volumes, such as 300 µL or 500 µL tubes, further contributes to resource conservation. Modular and scalable racks and tubes minimize storage space and reduce energy consumption during sample preservation by up to 80%. These features make LVL SAFE® products a sustainable solution that meets the growing demands of modern storage concepts.

FINAL SUMMARY AND EVALUATION AT A GLANCE

This technical documentation demonstrates – based on comprehensive testing – the outstanding suitability of LVL SAFE® 2D Tubes for use in modern biobanks, sample archives, and analytical and diagnostic applications requiring the highest standards of sample stability, purity, and process reliability.

The tubes meet the core requirements of international standards, including DIN 13279, ISO 20070, ISO 14644, ISO 11137, as well as test methods defined in Ph. Eur. 2.6.1 and USP <71>. The combination of a well-thought-out design, high-grade polypropylene (SAFE®-PP), and precision manufacturing under GMP conditions results in excellent sealing performance, chemical inertness, and biocompatibility. Production at LVL technologies is carried out under a certified quality management system in accordance with ISO 9001 and ISO 13485, ensuring consistent product quality and traceability.

LVL SAFE® 2D Tubes offer verified and documented quality across all relevant categories. This makes them ideally suited for both the long-term cryogenic storage of biological samples and the safe handling of chemical substances in automated systems. Their validated safety and purity ensure the integrity of sensitive samples and provide the foundation for reliable, reproducible results in research and diagnostics.

TECHNICAL SUPPORT AND DOCUMENT ACCESS

For comprehensive technical support and maximum transparency, we provide centralized online access to all relevant documentation for LVL SAFE® 2D Tubes and Racks. This ensures that you always have access to up-to-date materials for your quality documentation, validation, or practical implementation.

Available resources include

- Certificates on product quality, purity, and compliance with standards
- Technical drawings with all relevant dimensions and compatibility specifications
- Application guidelines for safe and efficient use in various fields

Access the download area
www.lvl-technologies.com/downloads

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