Cryo Competence Center LIFE SCIENCES

ILK Dresden

Insights on the tightness and long-term storage suitability of cryovials and straws in biobanking

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Scope

- Common use of cryovials/ -tubes and straws as packaging material for (long-term) storage and transport of biological samples (e.g. cell suspension, blood (components) or DNA)
- Storage above liquid nitrogen (reduction of biological degradation processes due to low temperature)
- Transport of frozen samples on dry ice (cost-effective, good availability)
- Transport via airplane (need to prevent leaking)

High demands on packaging materials: (long-term) sample integrity and user safety

- Protection against chemical and biological contamination
- Protection against sample loss due to leakage/ bursting

✓ Need to develop **test methods** under **practical conditions**
✓ Development of method for **artificial thermal aging**
✓ **Comparison** of fresh, aged and thermally aged tubes and straws

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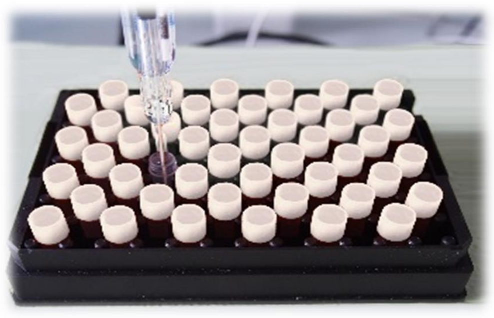
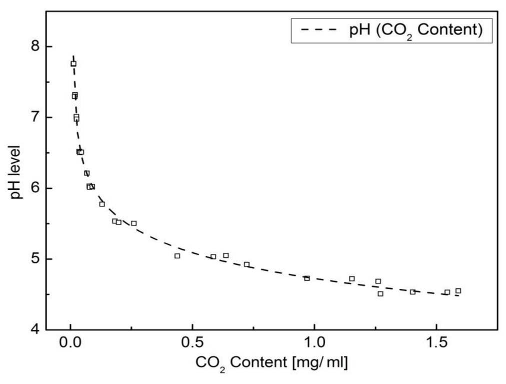
Materials & Methods

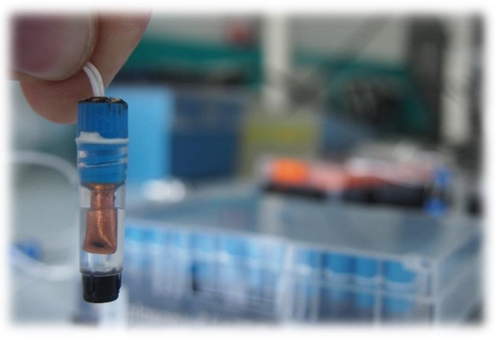
CO_2-Tightness Test ► Test of tightness for transport on dry ice



- Samples filled with 1mM Tris-HCl buffer and stored for **24h in saturated CO_2 atmosphere** over dry ice
- Measurement of pH value after thawing
- **Correlation of pH value with CO_2 content** in the buffer

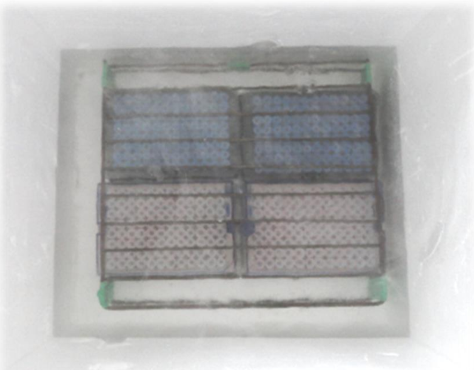


Gravimetric Tightness Test ► Test of tightness for airplane transport

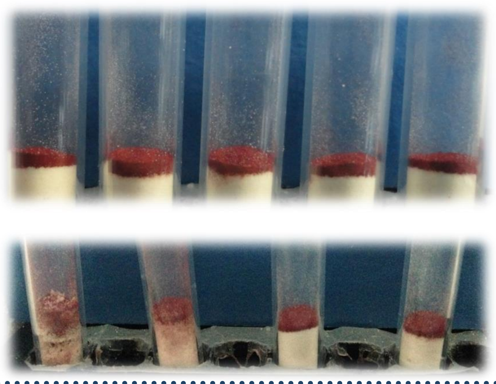


- Preparing tubes with equipment for thermal measurement
- Fill tubes with special anti-freeze
- Incubate tubes unter **pressure difference of 99 kPa** and temperatures from **−40 °C** to **+55 °C** with ΔT = 1 K/min
- Weigh before and after incubation

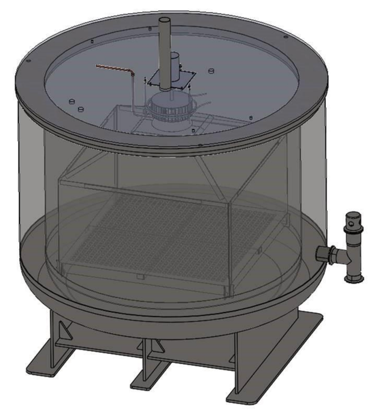
LN_2 Leak Test ► Test of tightness after direct contact with LN_2



- Fill tubes with two microgranulates
- Top layer azo dye bound in microgranular carrier substance
- Incubate tubes for 24 h in LN2
- Determine **visual mixing effect** and **residual pressure**



Artificial thermal ageing ► Development of thermal cycling chamber



- Temperature range: **−190 °C ≤ T ≤ 120 °C**
- Optimized airflow ΔT < 8 K (air temperature)
- Cooling up to 60 K/min
- Heating up to 10 K/min
- **Automated process** for thermal alternating load
- Only manual operation while filling LN2 supply

Measurement of thermal properties by DSC

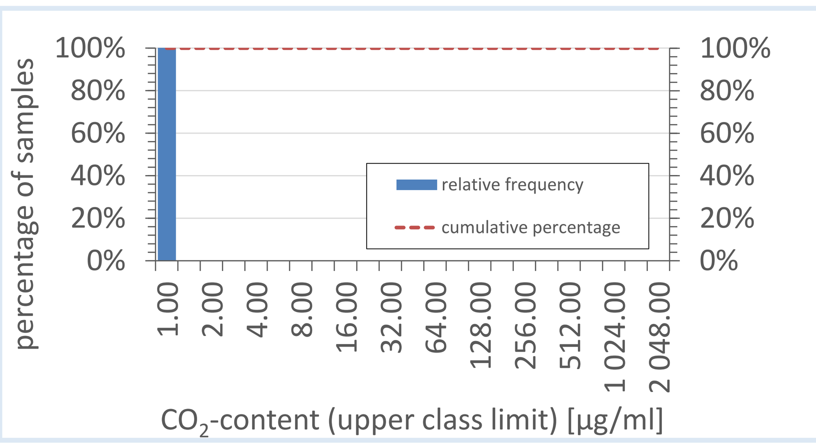
- Determination of:
  - Temperature and enthalpy of melting and solidification
  - **Glass transition temperature** and change in **heat capacity**
- Temperature program:
  - 1st Heating from −180 °C to 220 °C with 10 K/min
  - Cooling from 220 °C to -180 °C with 10 K/min
  - 2nd Heating from −180 °C to 220 °C with 10 K/min
- Parameter:
  - 8 to 10 mg of sample mass
  - 20 µl Al standard pan (non-hermetic)
  - Atmosphere: Helium 5.0

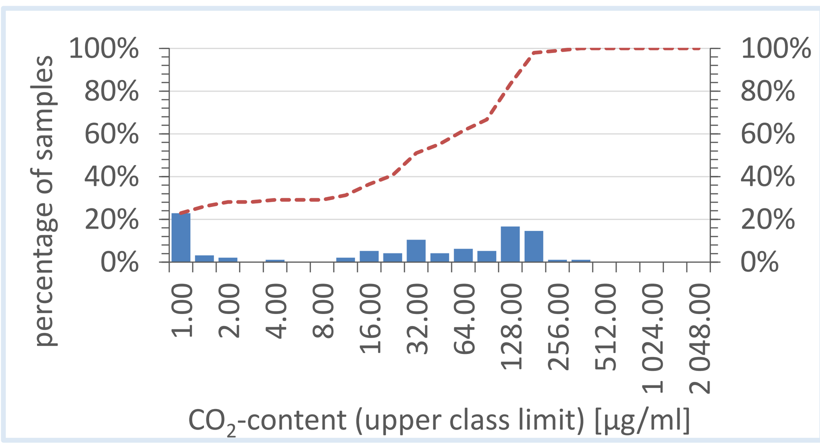
Measurement of mechanical properties by tensile tests

- Preparation of samples by cutting area of thread and foot
- Determination of maximum tensile force and calculation of tensile strenght
- Parameter
  - 2.5 kN tongs specimen holder
  - Clamping lenght between specimen holder 1 mm
  - Feed speed of 10 mm×min^{-1} at room temperature

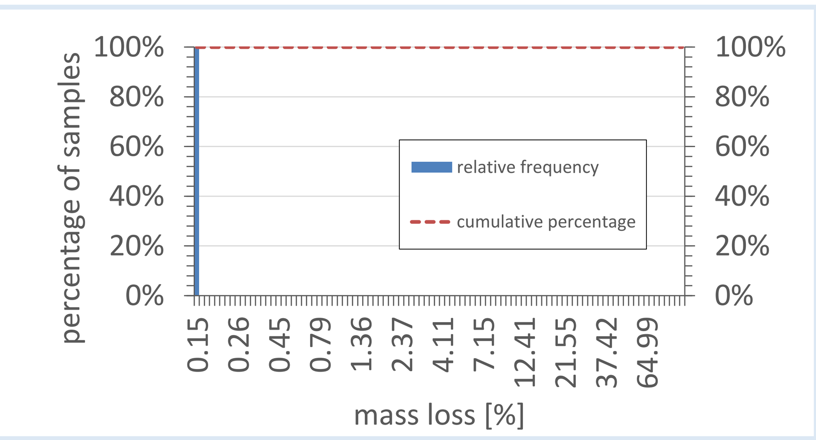
Results

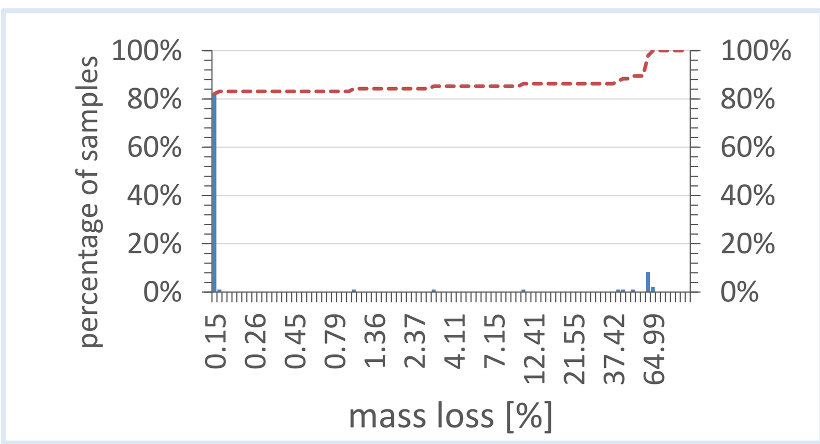
• CO_2 Tightness Test of fresh and thermally cycled* tubes Type C





• Gravimetric tightness test of fresh and thermally cycled* tubes Type C





➢ Some tubes show significant **decrease in tightness** test **after thermal cycling***
➢ Most of the tested tubes show no significant differences between tightness before and after thermal cycling

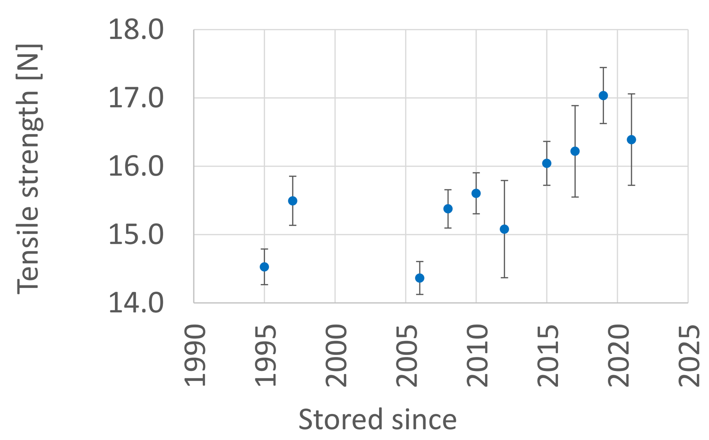
*Results shown were performed by manually cycling: 50 cycles with direct LN2 contact and rewarming to room temperature in temperature controlled drying oven at 60°C

• Changes in thermal properties of fresh and thermally cycled* tubes

Sample	Thermally cycled	Glass transition temperature T_g [°C]	Change in heat capacity Δc_p [J×g^{-1}×°C^{-1}]
A	yes	−17.4	0.124
A	no	−14.7	0.192
B	yes	−15.6	0.215
B	no	−12.5	0.151
C	yes	−13.2	0.241
C	no	−11.4	0.280
D	yes	−16.8	0.128
D	no	−12.9	0.141
E	yes	−18.1	0.302
E	no	−18.9	0.253

➢ No significant chances is melting and solidification peaks after thermal cycling
➢ No significant change in heat capacity
➢ Slightly **decreasing glass-transition temperature** for thermally cycled samples

• Decreasing mechanical properties with increasing storage time for Straws



➢ Noticable **loss of mechanical strength** due to **increasing storage time**
  ⇨ Loss of nearly 15% of tensile strength over 25 years of storage
  ⇨ Thermal stressed straws with **16.26 ± 0.59 N** show only a slight decrease in tensile strength

Conclusion

The developed methods for the tightness evaluation of cryovials and straws allow a differentiated and **objective evaluation of the tightness**.

➢ LN_2 leak test enables an evaluation of the **sample and work safety.**
➢ The amount of CO_2 in an absorption buffer can be **quantitatively determined**.
➢ The gravimetric tightness test allows **statistical evaluation** of the results.

- Thermal cycling of the packing material sometimes leads to **measurable changes in material structure and strength**.
- These material changes may cause **decreasing tightness** for some of tubes/ straws.
- **Sample reliability should be considered in the future** selection of the packing material.

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