Characterisation of thermal excursions in cryogenically stored vials: implications for exceeding the glass transition temperature of water

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Biological samples stored below −150°C in liquid nitrogen (LN$_2$) vapour-phase freezers rely on the assumption that biochemical and degradative processes cease below the glass transition temperature of water (Tg,$H_2O \approx -134$°C). However, transient temperature excursions during handling can expose samples to warming rates sufficient to exceed Tg,H_2O within seconds. This study characterises the warm-up behaviour of single H_2O-filled cryovials during typical handling conditions and quantifies the risk of crossing Tg,H_2O. Results from experimental measurements and finite element simulations are used to inform best practices for safe cryogenic sample handling.

Introduction

Cryogenic storage is a cornerstone of long-term biological sample preservation. The critical threshold for sample stability is the water glass transition temperature (Tg,H_2O), below which molecular mobility and chemical activity are effectively halted. However, during handling - particularly transfers from LN$_2$ storage to ambient (RT) or −80°C dry ice environments - samples are susceptible to rapid thermal excursions. Understanding the dynamics of vial warm-up rates is crucial to mitigate degradation risk during such events.

Materials and Methods

Experimental Setup

- **Samples:** FluidX™ 1.0 mL and 2.0 mL vials, and Wheaton™ 2.0 mL vials, filled with 100% or 25% of maximum working volume (MWV) with deionised H_2O.
- **Storage:** Vials equilibrated in a vapour-phase LN$_2$ freezer at −173°C.
- **Measurement:** Vials instrumented with three 32-gauge Type T thermocouples positioned at:
  1. 2 mm below the water surface
  2. Vial centre
  3. Vial base
- **Warm-Up Conditions:** Vials warmed by suspension in ambient air or immersion in dry ice pellets; no user contact. Warm-up occurred under natural convection unless otherwise noted.

Simulation Setup

Finite element (FE) models were developed in ANSYS 15.0 and calibrated against empirical data. Ice properties were temperature-dependent; polypropylene vial properties were assumed at room temperature.

Results

Warm-Up Rates

- **Single Vials (−175°C to −120°C):** Warm-up rates ranged from 55 to 255°C/min.
- **Inside Cryoboxes:** Rates were reduced to 5.4 to 66.7°C/min.
- **Time to Exceed Tg,H_2O:**

o **RT Environment:** 24–40 s

o **Dry Ice:** 9–45 s (faster due to enhanced conduction and CO$_2$ convection)

- **Volume Effects:** Smaller H_2O volumes warmed 15–35% faster, but volume sensitivity was modest compared to environmental impact.
- **Thermal Uniformity:** In both environments, spatial temperature variation in vial interiors was ≤4°C at any time, confirming near-uniform thermal distribution.

Table 1. Single tube warm-up rates[1] from -175°C storage to lab bench (21°C) or dry ice temperature [°C/min]

H20 filled tubes	FluidX 1.0 ml vial	FluidX 2.0 ml vial	Wheaton 2.0 ml vial
RT, 100% MWV	94 – 101	55 – 62	57 – 62
RT, 25% MWV	101 – 112	67 – 72	70 – 76
Dry ice, 100% MWV	171 – 213	92 – 120	100 – 143
Dry ice, 25% MWV	195 – 255	106 – 141	164 – 192

1. Linearised warm-up rate in the -175°C to -120°C range.

Table 2. Cryobox stored tube warm-up rates[1] from -175°C storage to lab bench (21°C) [°C/min]

H20 filled tubes	FluidX 1.0 ml vial[2]	FluidX 2.0 ml vial[3]	Biocision 2.0 ml vial[4]	Wheaton 2.0 ml vial[5]
Full rack, cover on	6.8 – 22.3	5.9 – 12.5	5.4 – 15.6	8.1 – 10.8
Partial rack, cover on	18.2 – 26.2	16.8 – 21.6	9.8 – 17.4	23.5 – 29.0
Full rack, cover off	15.0 – 36.9	13.0 – 19.8	6.9 – 20.9	10.2 – 24.3
Partial rack, cover off	36.2 – 50.4	28.5 – 66.7	11.5 – 25.8	34.9 – 44.0

1. Linearised warm-up rate in the -175°C to -130°C range. 2. 96 format storage tube with screw cap filled with 0.73 ml H20; perforated rack underside. 3. 9x9 sample storage tube with screw cap filled with 1.8 ml H20, no perforated rack underside. 4. 9x9 TruCool hinged cryobox with LN2 drain holes; tubes filled with 1.8 ml H20. 5. KeepIT-100 freezer box, tubes filled with 1.8 ml H20, perforated rack underside.

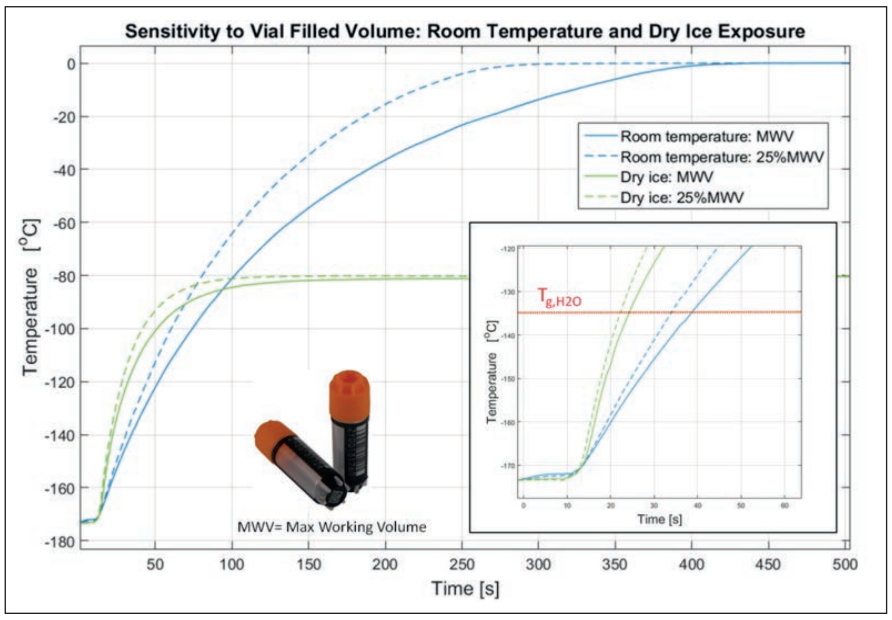

Figure 1: Warm-up rate sensitivity to volume and exposure environment. Lower H_2O volumes provide decreased thermal mass at equivalent heat energy absorbed by the vial. This corresponds to slightly increased warm-up rates (15 – 30%), yet the effect is not significant compared to a fast vial warm-up at T<-90°C. Single vials in dry ice warm above Tg,H_2O (-134°C) much faster than in an RT environment.

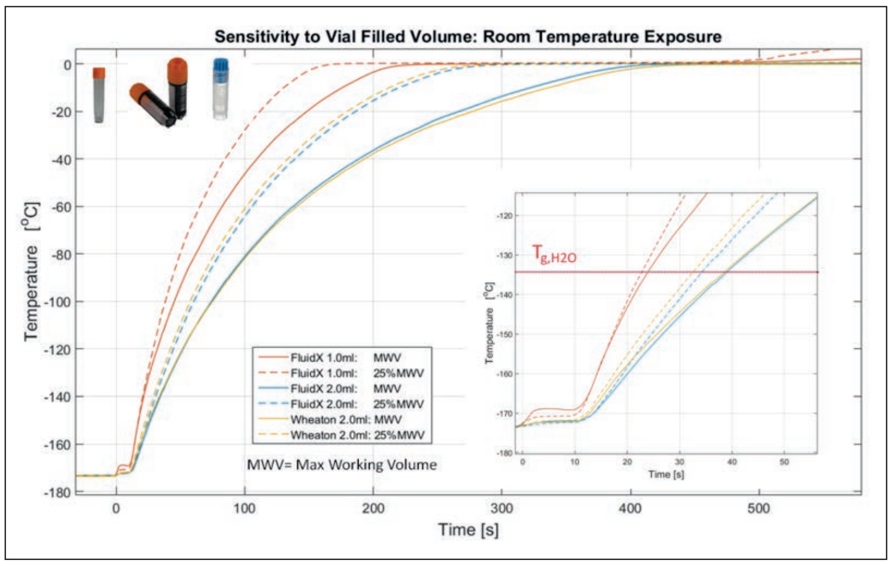

Figure 2: Vial warm-up rate sensitivity to H_2O volume in RT, interior sensor. Vials warmed to above Tg,H_2O at times ranging from 24 – 40 seconds. Initial temperature plateau detected in the -173°C to -169°C range is due to the short interval where vials sit inside the cryorack and cryobox when first extracted from the freezer. All vial interior sensors show comparable warm-up rate sensitivity to H_2O.

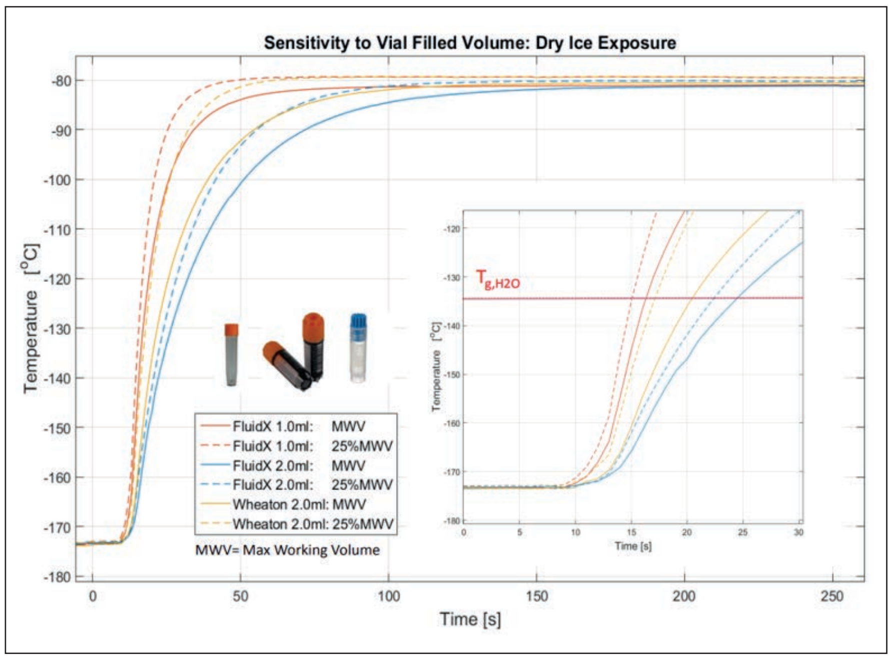

Figure 3: Vial warm-up rate sensitivity to H_2O volume in dry ice, interior sensor. Vials warmed to above Tg,H_2O at times ranging from 9 – 45 seconds. All vial interior sensors show comparable warm-up rate sensitivity to H_2O.

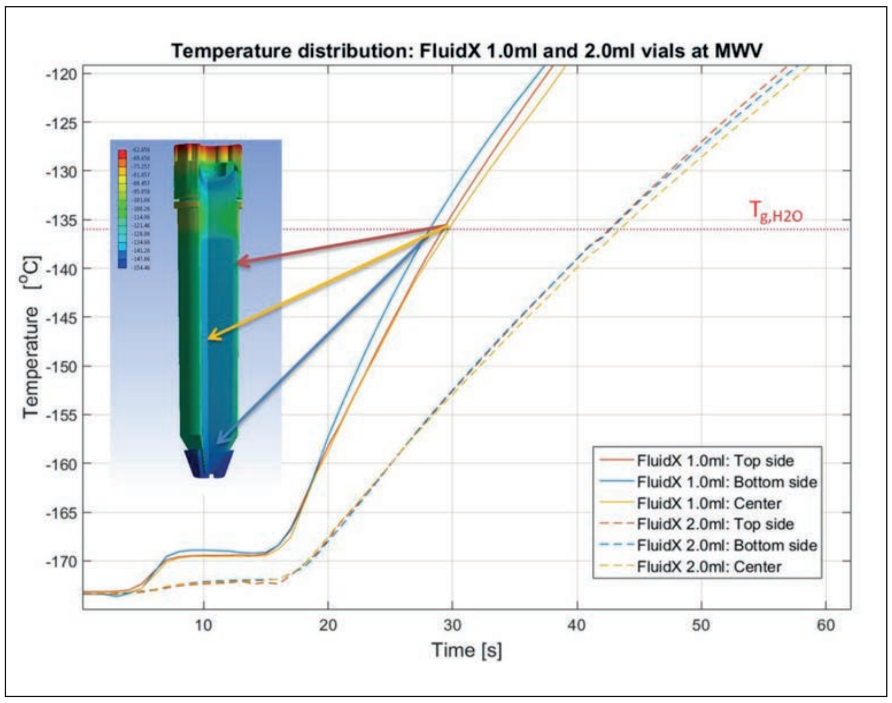

Figure 4: H_2O filled FluidX vial temperature distribution when extracted from cryostorage and exposed to RT. At any given time, the maximum spatial ice temperature variation is within 4 °C due to greater thermal diffusivity (α) of ice compared to polypropylene (PP) (e.g. αIce (T -150°C) = 1.07e-5 mm2/s vs. αPP (T 22 °C) = ~1.29e-7 mm2/s. Data indicates that H_2O temperature increase inside the vials is highly uniform with limited point-to-point spatial variations.

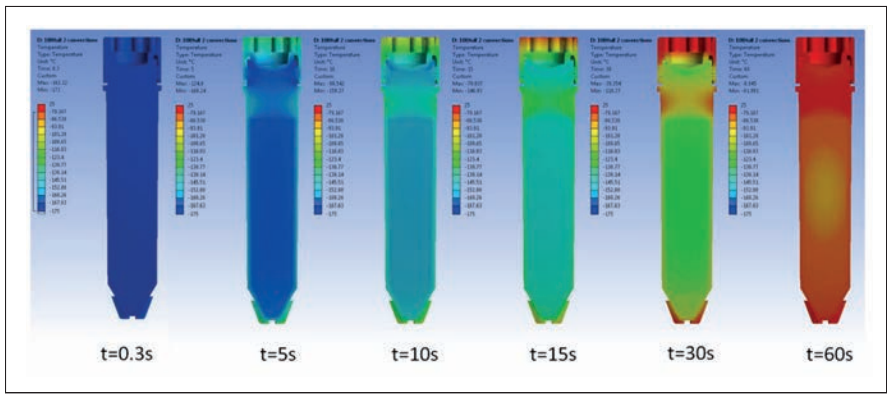

Figure 5: Simulated temperature distribution in a 100% MWV FluidX 1.0ml tube removed from cryostorage and exposed to RT conditions. Over time, the temperature is highly uniform throughout the entire sample volume.

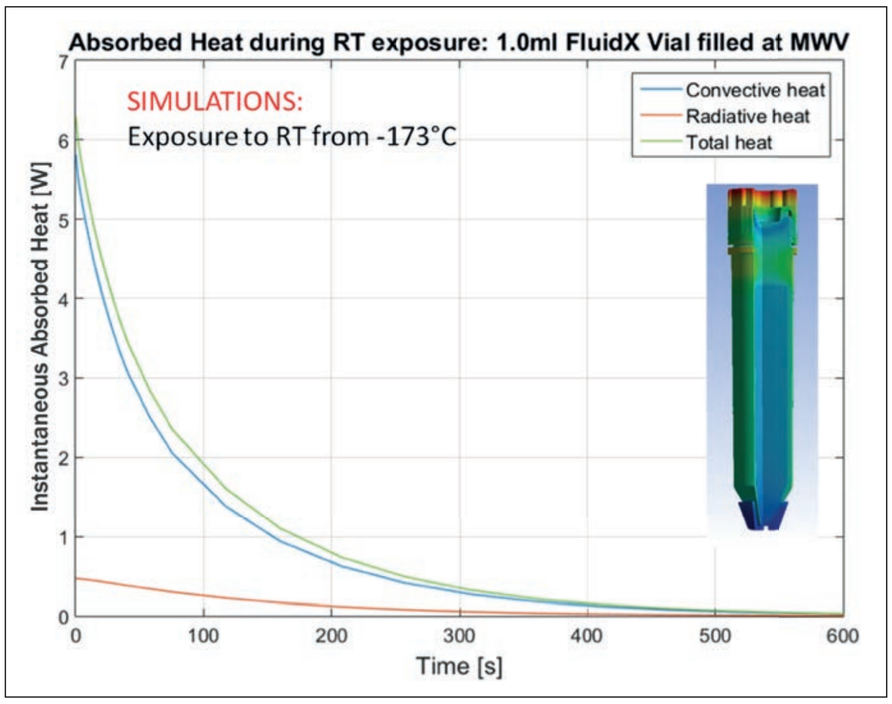

Figure 6: Simulated absorbed heat from the FluidX 1.0ml tube when warming from -173°C to -1°C. Convective heat transfer is responsible for the greatest contribution of energy transferred from the environment to the tube whereas conduction through the TC wires was negligible and thus not simulated.

Equation 1. Q=h•A•(Tenv−Tvial)•t

Handling vials in environments < −150°C reduces absorbed heat by >90%.

Equation 1: Calculation demonstrating how convective heat transfer into a vial can be greatly reduced by decreasing the environment temperature. For instance, handing a cryogenic vial in an environment below -150°C reduces the heat absorbed by the vial by more than 90%.

Simulation Insights

- Convective heat transfer dominated over conductive or radiative modes.
- Conductive heat through thermocouple wires was negligible.
- Simulated and measured warm-up profiles showed excellent agreement.
- Total absorbed energy could be reduced by >90% by maintaining handling environments below −150°C.

Discussion

The rapid approach to Tg,H_2O during even brief exposure to warmer environments underscores the need for stringent cryogenic handling protocols. Notably, dry ice environments may pose greater thermal risks than ambient air below −90°C due to conductive heat from direct contact and enhanced convection from CO_2 sublimation. Cryoboxes with lids significantly reduce exposure rates and should be used whenever possible. Handling time should be minimised, and, ideally, all manipulations should occur within environments maintained below −150°C.

Conclusion

Cryovials removed from LN_2 storage are at high risk of exceeding Tg,H_2O within seconds during handling. Warm-up rate is most strongly influenced by environmental temperature and vial exposure method, with minimal influence from vial volume or geometry. Cryogenic best practices should include:

- Keeping samples in cryoboxes with lids during handling.
- Limiting exposure time outside LN_2 environments.
- Performing handling operations in environments ≤ −150°C.

These steps help preserve biological sample integrity by preventing transient crossings above Tg,H_2O.