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# Standard biobanking conditions prevent evaporation of body fluid samples

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ABSTRACT

Pre-analytical variation in biobanking procedures, e.g., long-term storage, could confound biomarker outcomes. We investigated evaporation in various body fluids at different storage temperatures and storage durations.
Biobank sample tubes (Sarstedt 72.694.007) filled with water in different volumes (50, 100, 250, 500, 750, 1000, 1250, 1500 μl) were stored at different temperatures (−80 °C, −20 °C, 4 °C, room temperature (RT)) for 4.5 years and weighed at regular intervals. Next, saliva, serum, plasma, and CSF were stored in different volumes (50, 250, 500, 1000 μl) at different temperatures (−80 °C, −20 °C, 4 °C, RT) for 2 years. An extra set of CSF was stored in tubes with safe-lock cap (Eppendorf 0030 120.086) instead of a screw cap with o-ring.
No evaporation of water stored in biobanking tubes at −80 °C or −20 °C occurred over 4.5 years. Storage of saliva, serum, plasma, and CSF at −80 °C or −20 °C, monitored over 2 years, protected these samples from evaporation too. At 4 °C, evaporation was minor, approximately 1.5% (50 μl) or 0% (1 ml) yearly, where at RT it ranged from 38% (50 μl) to 2% (1 ml). Differences were observed neither between different body fluids, nor between tube caps.
Our data provide support for long-term biobanking conform current biobanking guidelines, encouraging retrospective use of clinical cohorts.



## 1. Introduction

Biobanks facilitate long-term storage of body fluids, such as blood, DNA, saliva and cerebrospinal fluid (CSF). Over the last years, there has been an increased interest in establishment of long-term biobanks, since biobanking has proven to be relevant for long-term clinical follow-up of patients and in addition enables research on new biomarkers for many diseases [1–5]. Long-term storage of body fluids at −80 °C has raised the question whether evaporation would occur within sample tubes. Evaporation of fluids could influence protein concentrations and thus is a relevant pre-analytical variation factor. Previous studies showed that the magnitude of evaporation in uncapped sample cups during 8 h at room temperature (RT) depends on several factors, including time and volume; environmental factors such as temperature, humidity, and air flow; and is additionally influenced by characteristics of the tube itself such as the geometry, design, and physical properties; and last but not least by the physicochemical properties of the fluid sample [6,7]. So far no experimental data exist on the effect of evaporation in closed sample tubes during long-term biobanking at freezing temperatures, except from our preliminary data presented in a review [8].

The aim of the current study was to evaluate whether evaporation is a pre-analytical confounder for long-term biobanking. We here report on an evaporation study with water, monitored for 4.5 years, and a second evaporation study in various body fluids, monitored for 2 years, both assessed in biobank storage tubes at different temperatures.

## 2. Materials and methods

### 2.1. Samples

Surplus serum, plasma and CSF from the routine diagnostic laboratory were used, according to the 'Research Code for Proper Secondary Use of Human Material' of the VU University Medical Center (VUmc) Amsterdam. Also, saliva, voluntarily donated by co-workers, and water were used.

*Abbreviations:* CSF, cerebrospinal fluid; RT, room temperature.

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### 2.2. Evaporation experiments

Water was divided into eight different starting volumes (50, 100, 250, 500, 750, 1000, 1250, 1500 μl) in Sarstedt tubes (Sarstedt AG, Nümbrecht, Germany; type 72.694.007) and stored at four different temperatures (−80 °C, −20 °C, 4 °C, RT) in duplicate. After thawing the tubes at RT and carefully drying their outside surfaces, evaporation was established by weighing the tubes using a semi-micro balance (Mettler Toledo, Columbus, OH, US; type AT250; calibrated yearly, measurement uncertainty set at 0.11 mg) at day 1 and after 1 week, 1 month, 1 year, 2 years and 4.5 years. Parts of the results have been published before [8].

In a second study, water, saliva, serum, plasma, and CSF were divided into four different starting volumes (50, 250, 500, 1000 μl) in Sarstedt tubes (type 72.694.007, with screw cap with o-ring) and stored at four different temperatures (−80 °C, −20 °C, 4 °C, RT) in triplicate. Evaporation was assessed as described above with measurements at day 3, and after 1 week, 1.5 month, 6 months, 1 year, 1.5 year and 2 years. Another set of CSF samples was stored in the same volumes and at the same temperatures in Eppendorf tubes with a safe-lock cap (Eppendorf type 0030 120.086) instead of a screw cap with o-ring. Tubes with and without screw cap with o-ring were selected for comparison based on the recommendations for biobanking [9]. Evaporation volumes were calculated using fluid densities, 1.0 g/ml for water and saliva, 1.025 g/ml for serum and plasma, and 1.0006 g/ml for CSF, and assessed absolutely as well as relatively [10,11].

### 2.3. Statistical analysis

We performed stepwise linear regression analyses with the relative change in volume as the dependent variable and storage temperature, time, starting volume, and in the second experiment also type of body fluid, as predicting factors. Data was processed using GraphPad Prism 6 Software (San Diego, California) and IBM SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA).

## 3. Results

The results in Fig. 1 show that no evaporation of water occurred when tubes were stored at −80 °C or −20 °C for 4.5 years. At 4 °C and RT we saw a decrease in volume over time, which was, expressed in percentage, higher in the small volumes than in the large volumes, 5% (50 μl) to 0% (1500 μl) at 4 °C, and 68% (50 μl) to 2% (1500 μl) at RT, after 2 years. In absolute numbers evaporation was on average 1.1 ± 0.03 μl a year at 4 °C and 2.5 ± 0.3 ml a year at RT. According to the linear regression analysis, temperature (β (SE) = 0.164 (0.014), $p < 0.0001$), time in years (β (SE) = 0.076 (0.011), $p < 0.0001$), and volume (β (SE) = −0.019 (0.007), $p < 0.01$) were significantly influencing this percentage volume loss.

The second experiment also showed no evaporation of water, saliva, serum, plasma, or CSF at −80 °C or −20 °C for up to 2 years, shown in Fig. 2. At 4 °C and RT we saw a decrease in volume over time, which was again in percentage higher in the small volumes than in the large volumes, 2% (50 μl) to 0% (1000 μl) at 4 °C, and approximately 80% (50 μl) to 4% (1000 μl) at RT, after 2 years. In absolute numbers evaporation was on average 0.5 ± 0.2 μl a year at 4 °C and 20.4 ± 1.6 μl a year at RT. According to the linear regression analysis, temperature (β (SE) = 1.932 (0.133), $p < 0.0001$), time in years (β (SE) = −3.557 (0.271), $p < 0.0001$), and volume (β (SE) = 0.004 (0.000), $p < 0.0001$) were influencing this percentage volume loss, while type of body fluid was no significant predictor for evaporation.

There was no difference in volume loss of CSF between the Sarstedt and Eppendorf tubes after 2 years, shown in Fig. 3.

## 4. Discussion

In this paper we show that water and body fluid samples stored at freezing conditions for up to 4.5 years are protected from evaporation, even for volumes as small as 50 μl. We performed two independent

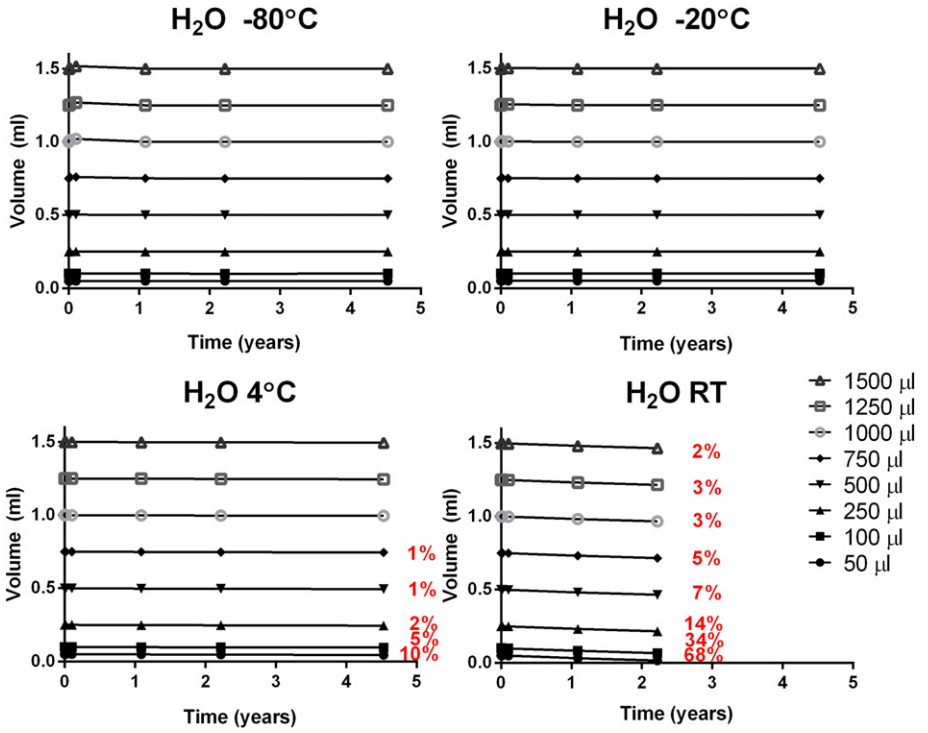


**Fig. 1.** Evaporation of water stored at −80 °C, −20 °C, 4 °C and RT in different volumes (50, 100, 250, 500, 750, 1000, 1250, 1500 μl) in Sarstedt biobank tubes over 4.5 years. The percentage of change was calculated and shown in red (only when values were ≥1%). Data up to two years were previously presented in a review [8]. Data points are means of two replicates, error bars (very small) are standard errors of the mean.