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Optimised sample handling in association with use of the CMA 600 analyser

Pernilla Abrahamsson*, Göran Johansson, Anna-Maja Åberg, Michael Haney, Ola Winsö

Department of Surgical and Perioperative Sciences, Anesthesiology and Intensive Care, Umeå University Hospital, SE-901 85 Umeå, Sweden

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ABSTRACT

A large degree of variability for batched analysis of serially collected microdialysis samples measured with the CMA 600 analyser has been described. This study was designed to identify sources of variability related to sample handling.

Standard concentrations of four solutes were placed in microdialysis vials and then stored and analysed at intervals. Results were analysed for variability related to vial and cap type, duration and temperature of storage, centrifugation and re-analysis.

The main results were that centrifugation of samples reduced variability. When a batch of 24 samples was analysed, the use of crimp caps reduced evaporation. Samples in glass vials with crimp caps could be stored in a refrigerator for up to 14 days without large variability in concentration compared to plastic vials which demonstrated variability already when stored for more than 1 day. We conclude that variability in microdialysis results can occur in relation to storage and analysis routines if routines are not optimised concerning evaporation. Centrifugation before analyses, glass vials with crimp caps even during frozen storage, and attention to minimal times for samples to be uncapped during analysis all contribute to minimise variability in the handling and analysis of microdialysis samples.

1. Introduction

Microdialysis is a mean of sampling substances from the body to help clinicians determine well being or metabolic conditions by providing serial biochemical samples from a catheter which lies within the substance of an organ [1–3]. Microdialysis sampling and analysis is very commonly employed for evaluation of physiological and pharmacologic scientific questions [4–11] with 3895 responses to Medline search for microdialysis until 2007. Still, there is uncertainty concerning variability in microdialysis results related to sampling and sample handling.

Current commercial microdialysis systems are equipped for sample collection, handling, and analysis of small molecules including glucose, lactate, pyruvate and glycerol as markers of cell injury or well being. Sample collection is based on passive diffusion through a semi-permeable membrane placed at the end of a catheter (in vivo) [1–3]. The CMA 600 analyser is a bedside analyser often used in clinical research. The CMA 600 analyser uses enzymatic reagents and colorimetric measurements of the microdialysis samples [12].

In previous experimental studies in our own laboratory, we have noted variability in results for microdialysis samples that were analysed in standard batches using the CMA 600 analyser. Specific aspects of sample handling and analysis routines may contribute to variability or error in results, and in particular there is concern about evaporation. Identification of sources of error and thus new strategies for elimination or reduction or error related to sampling would improve the precision and accuracy of microdialysis results. Therefore, we have set out to identify sources of variability in results related to sample storage, handling, and analysis. We specifically aimed to do this by analysing standardised microdialysis samples in batches related to vial type, capping and cap type before and during analysis, centrifugation, re-analysis, and storage conditions.

2. Materials and methods

Standardised solutions for glucose, lactate, pyruvate and glycerol, all in 'low', 'medium' and 'high' concentrations (Fig. 1) were used as 'test' samples. Each 'batch' comprised 24 samples (vials), and each vial was prepared with 20 μl of the respective standard solution. All samples were analysed in a CMA 600 analyser (CMA Microdialsysis, Solna, Sweden). All samples (except when specifically noted, in the case of refrigeration) were stored in the same freezer at −20 °C.

* Corresponding author. Tel.: +46 90 785 11 16; fax: +46 90 13 13 88.
E-mail address: pernilla.abrahamsson@anestesi.umu.se (P. Abrahamsson).

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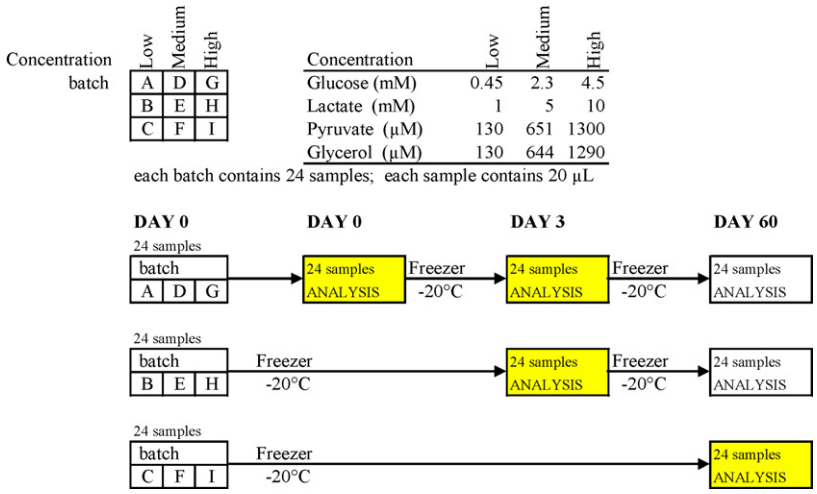


Fig. 1. Concentrations of different substances and how they were analysed, stored and re-analysed. Each batch contained 24 samples with 20 μl sample volume of low, medium or high standard solution of glucose, lactate, pyruvate and glycerol, respectively.

2.1. Drift and re-analyses

All solutions were prepared in batches using plastic vials (CMA, Microdialysis, Solna, Sweden) with same/single concentration in each whole batch. Each test started with immediate analysis of batch A0. After analysis, the samples were stored in a freezer for 3 days, and then re-analysed (A3). The same samples were then stored in the freezer for 60 days, and then re-analysed (A60). The same procedure was performed for the 'low'. 'medium', and 'high' concentrations of each substance.

2.2. Storage at −20 °C

Samples were placed in plastic vials without crimp caps. Two batches of each solution were prepared in vials on Day 0 and then stored in a freezer for 3 (B3, E3 and H3) or 60 days (C60, F60 and I60) before analysis. These results were compared with analyses on Day 0 (A0, D0 and G0).

2.3. Centrifugation

A group of samples prepared and handled in the same way as batch B3 was centrifuged (Mini Galaxy, VWR, West Chester, PA, USA) for 30 s before analyses. These samples were restored in a freezer for 60 days and then analysed again after centrifugation. These results were compared with results for analyses of B3 and B60.

2.4. Vial materials, different caps

One batch of 'low' concentration solution was prepared and placed in plastic vials with crimp caps (Chromacol, CT, USA), glass vials (Chromacol, CT, USA) with crimp caps, and microvials (CMA Microdialysis, Solna, Sweden). The vials were centrifuged for 30 s before analysis. After analysis, they were stored in a freezer for 3 days and then re-analysed.

2.5. Storage with plastic or glass vials and crimp cap vs microvials, freezer or refrigerator

Plastic vials and glass vials both with crimp caps (Fig. 2) were stored in a refrigerator or a freezer. Microvials were stored only in the freezer. Five batches containing 'low' concentration solution were prepared in plastic and glass vials. One batch from plastic and glass vials was analysed on Days 0, 1, 2, 3 and 14 after storage in a refrigerator. All samples were centrifuged before analysis. Batches with plastic vials, microvials, and glass vials were stored in a freezer for 70 days and then centrifuged and analysed.

2.6. Calculations and statistics

To calculate the relation of time for samples in each batch in the analysis machine to measured concentration (drift), linear regression analysis was performed (Fig. 3A). An estimation of the maximal time effect on concentration was presented as the difference between the intercepts of the regression line at time 0 and time at 192 min. The concentration change in percent (drift, %) was calculated as (absolute drift/*Y*-intercept) × 100.

To calculate variation we subtracted each sample value from the corresponding part of the regression line. We then added the start value, Y-intercept, to each calculated difference; this was functionally the same as placing the regression line, on the zero-level, and then adding the start value (Fig. 3B). This was done for both batches (Days 0 and 3) which enabled us to calculate the difference between mean concentrations for each batch (after correction for drift). Calculations of standard deviation (S.D.) and coefficient of variation (CV) for each batch were performed. Measured values are presented as mean ± S.D. Independent samples *t*-test between groups was used for testing for differences in mean concentration between two batches. A p value of less than 0.05 was used for statistical significance. The analyses were performed with the SPSS software package (version 14.0; SPSS, Inc., Chicago, IL, USA). The one-sample Kolmogorov–Smirnov test was used to determine normality in results distribution.

3. Results

All results presented are for samples with 'low' concentrations of glucose, lactate, pyruvate and glycerol with the exception of the first results section (drift and re-analyses) where there in addition were analysis for 'medium' and 'high' concentrations. In total, 1248 separate measurements were performed and analysed. All results demonstrated a normal distribution.

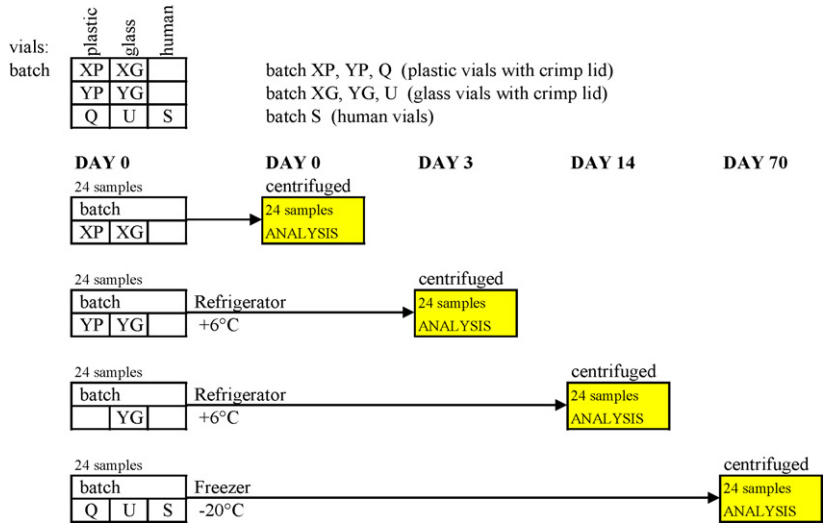


Fig. 2. Concentrations of different substances and how they were analysed and stored. Each batch contained 24 samples with 20 μl sample volume of low standard solution of glucose, lactate, pyruvate and glycerol, respectively.

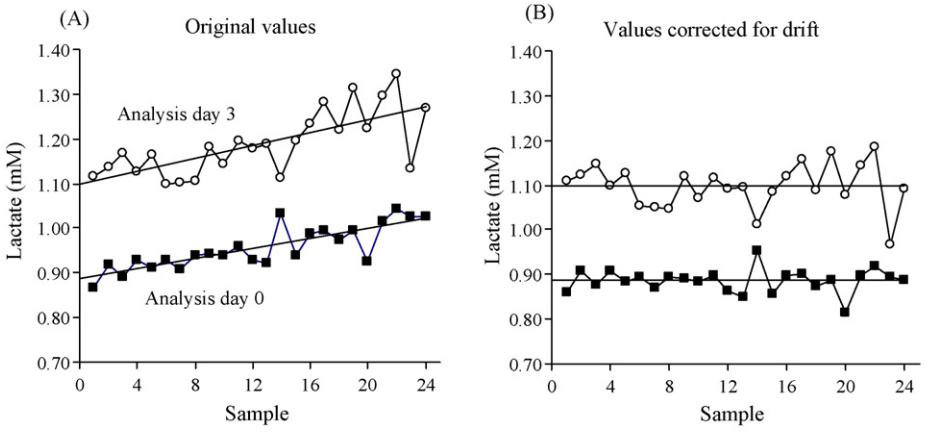
3.1. Drift and re-analyses

The temperature inside the CMA 600 analyser was measured to be 27 °C. Programmed batch analyses of 24 samples over 192 min showed a difference in concentration (drift) of 12–25% for the four substances (Table 1). Differences between mean concentrations range from 18% to 25%, with significantly higher values on Day 3 compared to Day 0 when re-analysing the same samples. The coefficient of variation (CV) between samples was higher for Day 3 compared to Day 0 for all substances. Similar results were obtained when analysing 'medium' and 'high' concentrations of the substances but they are not presented in the interest of clarity and brevity.

Table 1
Plastic vials without crimp cap (freezer 3 days).

Days in freezer	Mean concentration		CV (%)		Drift (%)	
	0	3	0	3	0	3
Plastic vials						
Glucose (mM)	0.39 ± 0.01	0.48 ± 0.02*	1.6	3.4	12.3	19.9
Lactate (mM)	0.89 ± 0.02	1.11 ± 0.04*	3.1	3.8	15.3	15.9
Pyruvate (μM)	109 ± 3.0	129 ± 5.8*	2.7	4.5	15.8	24.7
Glycerol (μM)	94 ± 2.9	111 ± 5.4*	3.1	4.9	17.0	22.6

*$p < 0.05$ using between groups t-test, mean ± S.D., coefficient variation (CV, %) and drift (%).



Linear regression: day 0 : Y=0.0073X + 1.098
day 3 : Y=0.0056X + 0.886

A	day 0	day 3		B	day 0	day 3	Conc-diff	
slope:	0.0056	0.0073		Mean concentration:	0.886	1.098	0.212	mM
drift:	0.135	0.174	mM	SD:	0.027	0.050		mM
drift%:	15%	16%		CV:	3.0	4.5		%

Fig. 3. Example of calculations made for each batch analysis of 24 samples, calculating the drift and spread of each batch and the difference in concentration between the two batches.

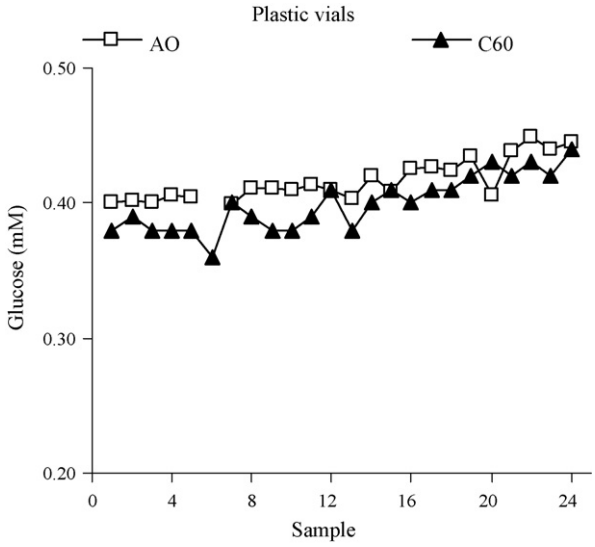


Fig. 4. This figure shows drift for one 24-vial batch of glucose (low concentration) on Day 0 (A0, 12.3% drift) and on Day 60 (C60, 16.3% drift) after storage in a freezer.

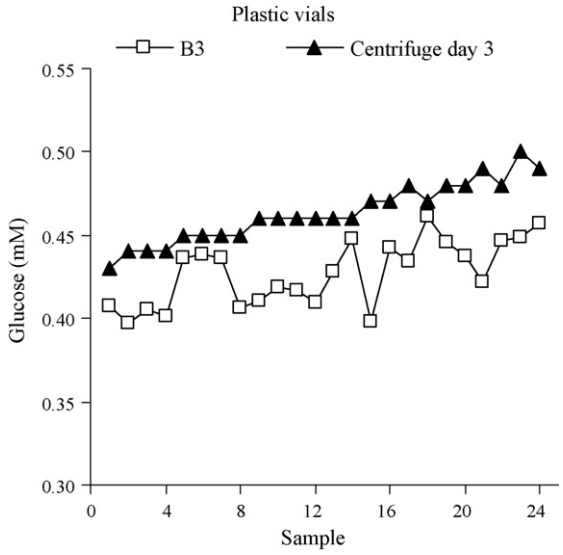


Fig. 5. Samples with glucose that were centrifuged (centrifuged on Day 3) compared to samples that were not centrifuged (B3).

3.2. Freezer storage (−20 °C)

There was no difference in concentration observed for any of the substances when the samples were stored in −20 °C for 60 days compared to Day 0 (Fig. 4).

3.3. Centrifugation

Variation between samples in one batch was reduced with centrifugation (Fig. 5). The CV for the samples without centrifugation (B3) was 3.6% (glucose), 4.3% (lactate), 2.6% (pyruvate), and 3.6% (glycerol), respectively. After centrifugation before the corresponding analyses on Day 3, the CVs were reduced to 1.0, 1.2, 1.4, and 2.8%, respectively.

3.4. Vial materials, different caps

Drift during batch analysis was the same between the different vials on Day 0 (Table 2). After 3 days in the freezer, samples in plastic vials with crimp cap and microvials had a larger change in concentration in glucose and pyruvate compared to those stored in glass vials with crimp cap. Also, there was an isolated change in lactate concentration in microvials at 3 days, −20 °C. The main finding here

Table 2
Plastic vials with crimp cap, microvials and glass vials with crimp cap (freezer 3 days)

Days in freezer	Mean concentration		CV (%)		Drift (%)	
	0	3	0	3	0	3
Plastic vials						
Glucose (mM)	0.42 ± 0.01	0.47 ± 0.04*	2.4	5.7	1.4	7.4
Lactate (mM)	1.04 ± 0.03	1.28 ± 0.08*	3.2	5.9	−2.0	0.6
Pyruvate (μM)	147 ± 3.4	165 ± 13.4*	2.3	8.1	2.8	11.0
Glycerol (μM)	132 ± 1.9	157 ± 6.9*	1.4	4.4	3.9	4.4
Microvials						
Glucose (mM)	0.39 ± 0.004	0.50 ± 0.02*	1.1	3.6	0.1	−11.4
Lactate (mM)	0.95 ± 0.02	1.17 ± 0.04*	2.5	3.4	3.4	−11.7
Pyruvate (μM)	142 ± 2.3	162 ± 5.8*	1.6	3.6	0.8	−11.9
Glycerol (μM)	129 ± 1.9	139 ± 5.2*	1.5	3.8	3.9	4.4
Glass vials						
Glucose (mM)	0.44 ± 0.01	0.46 ± 0.01*	1.9	2.1	−0.2	4.9
Lactate (mM)	0.99 ± 0.04	1.13 ± 0.02*	4.2	1.6	−0.6	2.5
Pyruvate (μM)	147 ± 2.5	161 ± 3.5*	1.7	2.2	−2.1	5.4
Glycerol (μM)	121 ± 2.6	145 ± 5.1*	2.1	3.5	0.3	−2.8

*Sign $p < 0.05$ using between groups t-test, mean ± S.D., coefficient variation (CV, %) and drift (%).

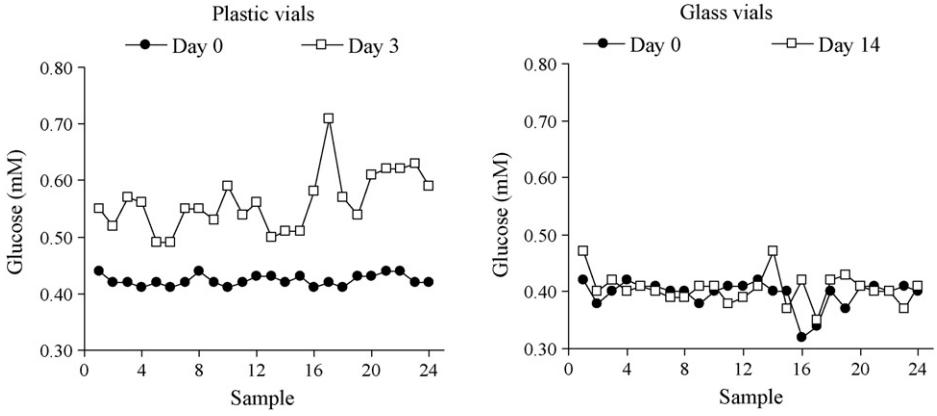


Fig. 6. Analyses of glucose. The left panel shows the results from samples analysed on Day 0 and samples stored in plastic vials in a refrigerator for 3 days. The right panel shows the results from samples analysed on Day 0 and samples stored in glass vials in a refrigerator for 14 days.

Table 3
Long- term freezer storage, plastic vials with crimp cap, microvials and glass vials with crimp cap

Day	Drift (%) 70	Difference (%) 0/70	CV (%) 70
Plastic vials			
Glucose (mM)	31.0	88	33.7
Lactate (mM)	29.5	96	35.0
Pyruvate (μM)	31.7	77	28.7
Glycerol (μM)	31.9	94	31.3
Microvials			
Glucose (mM)	−1.5	11	7.6
Lactate (mM)	−1.3	13	10.0
Pyruvate (μM)	−0.3	4	4.2
Glycerol (μM)	−3.7	−6	5.9
Glass vials			
Glucose (mM)	−0.3	−6	2.5
Lactate (mM)	−1.9	22	5.3
Pyruvate (μM)	−2.6	6	2.0
Glycerol (μM)	−2.1	9	1.6

Coefficient variation (CV) (%).

was that re-analysis of all samples was associated with higher concentrations. The largest difference (30%) between samples analysed on Day 0 and then re-analysed on Day 3 was for glucose stored in microvials. The corresponding difference for glucose stored in glass vials with crimp cap was only 5%. There was less drift in plastic vials with crimp cap on Days 0 and 3 compared to the same vials without crimp cap Days 0 and 3 (Table 1). Notably, there was apparent minus drift (approximately 11%) for three of the four substances in microvials suggesting dilution or water addition to the samples.

3.5. Storage with plastic or glass vials and crimp cap vs microvials, freezer or refrigerator

Microdialysis samples in plastic vials with crimp cap demonstrated large changes in concentrations when they were stored for 3 days in a refrigerator (+4 to 6 °C) (Fig. 6, left panel). Glucose results are shown in Fig. 6 (right panel), and all four substances were unchanged in concentration in glass vials with crimp cap for up to 14 days in the refrigerator.

The results of freezer storage at −20 °C for 70 days (Table 3) showed that the variation was related to type of vial and cap. Samples in plastic vials with crimp cap showed increases in mean concentration of 77–96% after freezer storage. The corresponding mean concentration difference for glass vials ranged from −6% to 22% and for microvials 6–13%. The size of the CV differed in relation to type of vials: plastic vials 29–35%, microvials 4.2–10.0%, and glass vials 1.6–5.3%.

4. Discussion

Of the effects of sample handling and storage on the reproducibility of microdialysis results, we found large differences in measured sample concentrations related to specific steps in sample handling that were alarming. This is new information, and improved understanding of systematic sources of error related to sampling should help investigators using this method improve their reproducibility and certainty concerning producing results. We initiated this study due to concern particularly for sample disturbances related to storage of microdialysis samples, but the findings were that storage in a freezer by itself did not affect the microdialysate. Unexpectedly, we found that the main contributor to variability in results occurred during longer batch analysis. With this study design, analysis of one batch occurred over 192 min, and the temperature in the analyser was 27 °C. These conditions are likely to be common among investigators who employ microdialysis methods.

Steps in the sample handling and analysis process which clearly contributed to variability included plastic vials with crimp cap, caps which have been penetrated for previous analysis for all vial types, and absence of centrifugation. Of these, the prominent negative influence of the drift on variability during batch analysis was most unappreciated by us before the study.

The process which led to drift was evaporation. Evaporation is perhaps unavoidable, but steps can be taken to minimise its effects on microdialysis samples. Evaporation occurs in direct relation to water exposure to air, temperature and the partial pressure of water in the air. We presume evaporative processes here, though we have not measured evaporation directly. Steps which limit evaporation include restriction of contact of water with surrounding air, and all aspects of the microdialysis sample environment are relevant. Vial material probably is not a factor here when considering plastic or glass vials, but, the degree of air-tight seal between the vial and the cap is very relevant, as shown by results for plastic vials and subjectively imperfect seals with crimp caps. Also, caps which allow analysis by penetration of a needle in the analysis machine but remain in place were demonstrated in our results to be not air tight afterward.

A notable secondary finding was that there seemed to be concentration decreases indicative of possible water capture for samples place in the freezer. This finding warrants further investigation, but if true, then evaporation is not the only source of variability related to water transit and microdialysis samples. These preliminary results allow speculation that water vapour is entrapped in the vials during freezing, leading to dilution at analysis.

Alternative mechanisms include possible alteration of total amounts of measured substances in solute during sample handling. This study was not designed to identify specific mechanisms of concentration changes, and another study design would be needed to confirm or refute these, and one area of focus could be to follow amounts of water as well as solutes through the sample handling process.

There are very few published results for reproducibility in microdialysis related to sample handling and storage. In an earlier investigation of microdialysis results variability performed in our lab, it was noted that 48 min analysis time (for glucose) for a batch in the same apparatus produced results with minimal variability [12]. Drift during analysis occurred for 192 min batches, and where significant drift may begin to occur had not been defined by our results. The manufacturer has issued some guidance concerning sample handling. The product description for the CMA Microdialysis system recommends that microdialysis samples should be stored in a −70 °C freezer. This recommendation is based on the observation that pyruvate concentrations in microvials already after 40 days in a −20 °C freezer were reduced by 15%. However, in our study when samples were stored in a −20 °C freezer for 70 days, pyruvate samples concentration in microvials and glass vials with caps were 4% and 6% higher compared to values on Day 0 (shown in Table 3), which is expected if a small amount of evaporation occurs. Our findings suggest that pyruvate sample integrity can be maintained in a −20 °C freezer for over months as long as they are stored in microvials or glass vials with crimp cap. At the same time, we observed increases in concentrations in lactate both in microvials and glass vials with crimp cap after 70 days in the freezer.

Concerning study design and limitation we chose standardised start concentrations as a surrogate for assessment of evaporation rather than sample weight. This was because we judged weight measurement in frozen microdialysis vials to be too imprecise as a specific assessment of sample volume. In future studies, weight

might provide additional support for assessment of evaporative events, and should be considered as a supplementary method. Concerning vial materials and matching of vials and caps, we did not test plastic vials with plastic caps. This was because we limited our study to vials and caps which could be placed directly into the analyser. Plastic caps must be changed to another cap (crimp cap, if a cap is to be used) before larger batch analysis. We noted beneficial results for centrifugation, and presume that this was related to reduction of microbubbles in the solutions. Presence of microbubbles in samples was not tested directly, and this mechanism for benefit needs to be confirmed.

Our findings have clinical implications which have led us change our routines concerning microdialysis sample handling and analysis with the CMA 600 analyser. Implications are that optimisation of storage with minimal dehydration related to storage time or temperature and minimising of evaporation during the analysis process are needed for all applications of microdialysis where evaporation can occur. Other implications based on our findings include the following. All samples that are stored before analysis should be centrifuged. Glass vials with crimp cap should be used as often as possible (non-clinical settings) since they are more robust in resisting evaporative processes. Samples collected in glass vials with crimp cap are considered "consumed" after analyses because significant evaporation occurs later if the crimp caps have been penetrated. 70 days storage at −20 °C as well as glass vial with crimp cap storage for 14 days at 4–6 °C do not by themselves lead to significant evaporation. Finally, all microdialysis samples in any specific study should be handled and analysed in a standardised way.

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