

Investigating the Effects of Lyophilisation on General Serum Chemistry EQA Material for Routine Measurands

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Introduction

The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) in association with the Australasian Association of Clinical Biochemists (AACB) have previously demonstrated commutability in a select range of endocrine measurands when base pool serum has been lyophilised¹ and fortified².

The source material for the RCPAQAP General Serum Chemistry and Therapeutic Drugs (GC) EQA program is subject to various forms of manipulation (dialysis, filtration, fortification and lyophilisation) to produce the desired product. Each stage in this production cycle potentially introduces changes in the base material such as protein and lipid denaturation which may lead to non-commutability.

The RCPAQAP intend on conducting a broad study to characterise the GC EQA material, exploring the effect each stage of production has on the degree of commutability. This initial project sought to determine the impact of lyophilisation on a sub-group of routine biochemistry measurands: ALT, ALP, amylase, calcium, creatinine, glucose, potassium and urea.

Method

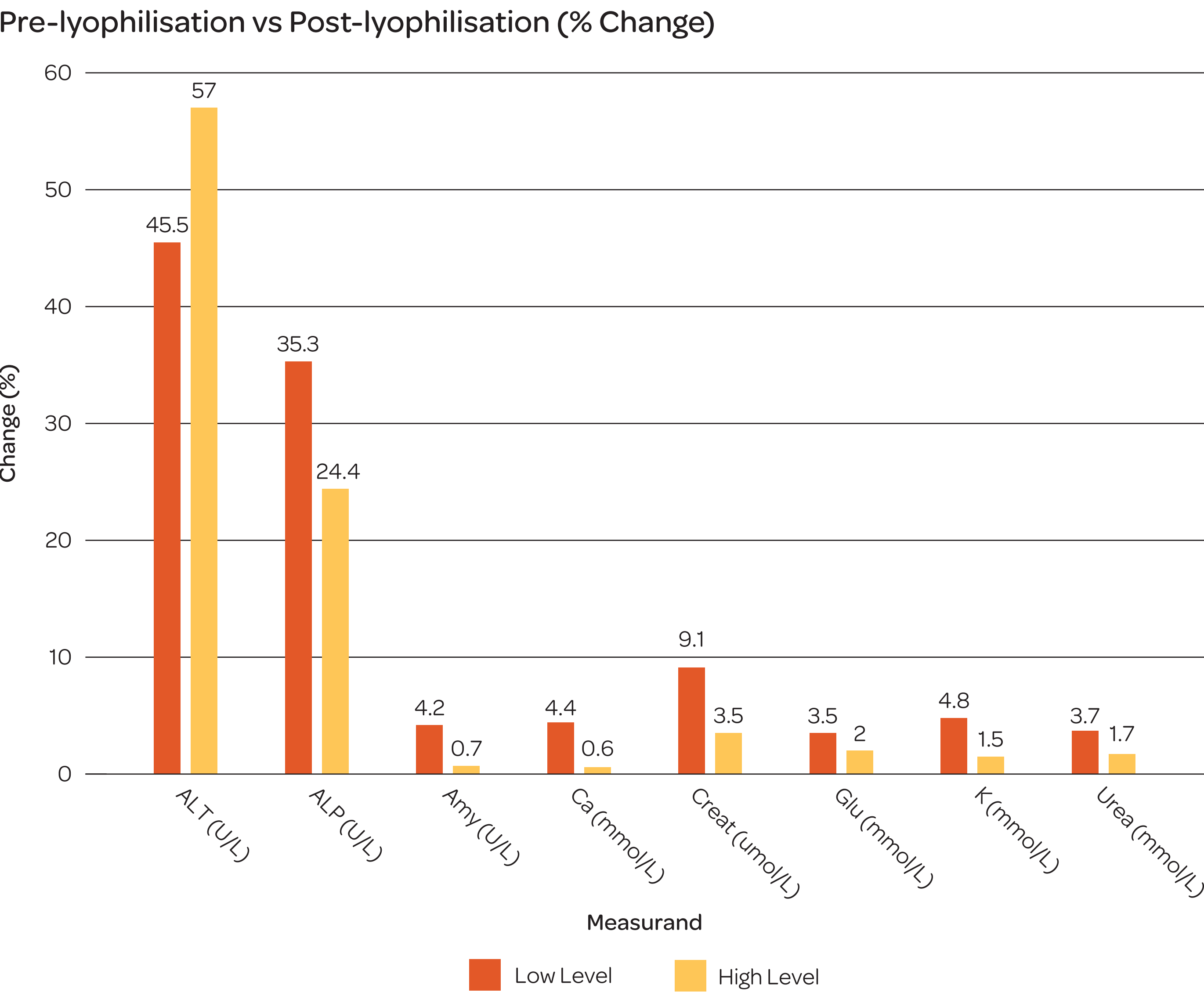
Pre-lyophilised aliquots of the 2018 GC material were obtained from the manufacturer and stored at -30°C. A low and high pool sample was distributed to 26 volunteering laboratories, with the following instruments represented: Abbott Architect c4000/c8000/c16000, Roche Cobas c501/c502/c701/c702, Ortho Clinical Diagnostic VITROS 4600/5600, Siemens ADVIA/Chemistry XPT, Siemens Dimension EXL, Beckman Coulter AU 480/680 and Beckman Coulter UniCel DxC 800. Participants were requested to analyse each sample for routine biochemistry measurands (note: not all instruments were tested for all measurands). The returned results for each laboratory were compared to their corresponding lyophilised sample result previously submitted for cycle 107, in the 2018 GC program. Imprecision was assessed by calculating standard deviation (SD) and analytical coefficient of variation (CVa). Changes in recovery from pre-lyophilised to post-lyophilised was calculated and expressed as a percentage.

Results and Discussion

Table 1: Summary of results from 26 participating laboratories comparing pre-lyophilised (Pre-Ly) to post-lyophilised (Post-Ly) recoveries for both low and high samples. Imprecision (SD and CVa) was calculated from the dataset and the average change expressed as a percentage (% diff mean).

		ALT (U/L)		ALP (U/L)		Amy (U/L)		Ca (mmol/L)		Creat (umol/L)		Glu (mmol/L)		K (mmol/L)		Urea (mmol/L)	
		Pre-Ly	Post-Ly	Pre-Ly	Post-Ly	Pre-Ly	Post-Ly	Pre-Ly	Post-Ly	Pre-Ly	Post-Ly	Pre-Ly	Post-Ly	Pre-Ly	Post-Ly	Pre-Ly	Post-Ly
Low	median	15.5	20.5	46	28	44	46	1.43	1.50	41	43	1.9	1.9	2.0	2.1	2.1	2.2
	SD	2.8	4.5	3.4	3.2	1.7	2.6	0.03	0.03	5.9	5.2	0.07	0.1	0.1	0.1	0.1	0.2
	CVa	19.5	21.1	7.5	10.9	3.9	5.8	2	2.2	14.8	12.1	3.9	4.4	4.2	3.2	6.5	7.7
	% diff mean	45.5		- 35.3		4.2		4.4		9.1		3.5		4.8		3.7	
High	median	112	179	592	447	441	442	3.30	3.29	322	313	25.5	24.9	6.6	6.5	22.9	22.3
	SD	11.5	14.6	32.3	36.1	13.7	12.6	0.08	0.09	22.2	17.5	0.72	0.8	0.1	0.1	0.4	0.7
	CVa	10.1	8.2	5.5	8.2	3.1	2.8	2.3	2.8	6.7	5.5	2.8	3.1	1.5	1.4	1.8	3.1
	% diff mean	57		- 24.4		0.7		- 0.6		- 3.5		- 2.0		- 1.5		- 1.7	

Figure 1: Mean difference in recovery expressed as absolute percentage change.



The mean difference (%) between the pre-lyophilised and post-lyophilised recoveries ranged from 45.5-57.0 for ALT, 24.4-35.3 for ALP, 0.7-4.2 for amylase, 0.6-4.4 for calcium, 3.5-9.1 for creatinine, 2.0-3.5 for glucose, 1.5-4.8 for potassium and 1.7-3.7 for urea.

Generally, the low sample saw minimal increases in recovery, with a median difference of +4.3% whereas the high sample saw minimal losses with a median difference of -1.6%. When the RCPAQAP analytical performance specifications are applied to the pre-lyophilised median result, the mean difference (% diff mean) fell within specifications for amylase, calcium, creatinine, glucose, potassium and urea, suggesting good correlation. More often than not, analytical precision was better the in pre-lyophilised samples compared to post-lyophilised. The need to reconstitute the post-lyophilised samples likely contributes to this imprecision. The low recovery for ALT and ALP in the pre-lyophilised samples was an unexpected finding and may be a result of deterioration due to prolonged storage at -30°C. It is understood that liver enzymes are over-supplemented in the GC material to compensate for losses during freeze-drying.

Conclusion

The results of this study would indicate that lyophilisation has minimal impact on the recovery and commutability of amylase, calcium, creatinine, glucose, potassium and urea in this EQA material. Liver enzymes displayed greater variability and require further investigation at lower storage temperatures (e.g. -80°C).

References

- ¹ Koetsier S, Jones GRD et al. Effect of lyophilisation on measurement of selected endocrine analytes – an RCPAQAP study. 2017.
- ² Shepherd S, Jones GRD et al. Commutability of a selection of endocrine analytes in native and spiked serum samples. 2018.

Acknowledgements

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