

Cool Temperatures, Hot Topic: Renin Cryoactivation at -20°C

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Introduction

Measurement of plasma renin concentration is required to calculate the aldosterone renin ratio (ARR), a screening test for primary hyperaldosteronism.

Cryoactivation (in vitro conversion of prorenin to renin at cold temperatures) is a specific challenge for renin assays, known to occur when plasma samples are stored between -5 and +4°C.^{1,2} Cryoactivation will result in an artefactually increased renin concentration, lowering the ARR. This may lead to patients with primary hyperaldosteronism being falsely reassured by a normal ratio, a missed diagnosis, and lost opportunity to treat a potentially reversible condition.

Results from a previous study suggest that storage at -20°C may not prevent cryoactivation.³ We investigated the extent of renin cryoactivation at -20°C in comparison to storage at -80°C, and renin stability over five days under the two storage conditions.

Method

Twenty patient samples (EDTA blood) collected for routine renin measurement were used. Samples were kept at room temperature and separated within two hours. The clinical indication for the patient samples included adrenal incidentaloma, pre- and post- saline suppression test, and secondary hypertension screen.

Renin was measured by direct renin chemiluminescent immunoassay on the Immunodiagnostic Systems iSYS platform. A baseline room temperature measurement was taken and then two aliquots were made from each sample, for storage at -20°C or -80°C. Renin was measured in the aliquots seven days later.

A renin stability study was also performed using EDTA blood from five volunteers. Samples were separated within one hour of collection and baseline room temperature renin results were recorded. Aliquots were then made for storage at -20°C or -80°, and tested over five days, with each aliquot undergoing only one freeze-thaw cycle.

Results

After storage at -20°C for seven days, renin concentrations changed between -5.2 to +76.6 mIU/L from baseline. The samples stored at -80°C demonstrated a change from -4.2 to +5.1 mIU/L from baseline (Figure 1). Cryoactivation was particularly notable in specimens #19 and #20, with results increasing by 76.6mIU/L and 57.8mIU/L respectively in the specimens stored at -20°C.

The RCPAQAP Analytical Performance Specifications (APS) for renin are +/- 6mIU/L for samples up to 20 mIU/L or +/- 30% for samples over 20 mIU/L. Seventeen of the twenty patient samples stored at -20°C met the APS, while all of the twenty samples stored at -80°C met the APS. Of note, only three samples had a renin concentration greater than 20mIU/L at baseline.

The stability study in the volunteer renin samples also demonstrated marked variation from baseline in the -20°C storage conditions (Figure 2). The renin results ranged from 20.2 to 41.7 mIU/L at baseline.

One sample increased by 124.6 mIU/L from baseline on day 3, while the following day an aliquot from the same volunteer stored under the same conditions had only increased 2.9 mIU/L from baseline. All of the aliquots stored at -80°C showed much less variation and stayed within the RCPAQAP APS.



Figure 1: Difference in direct renin concentration following storage at -20°C or -80°C. One outlier was removed for clarity.

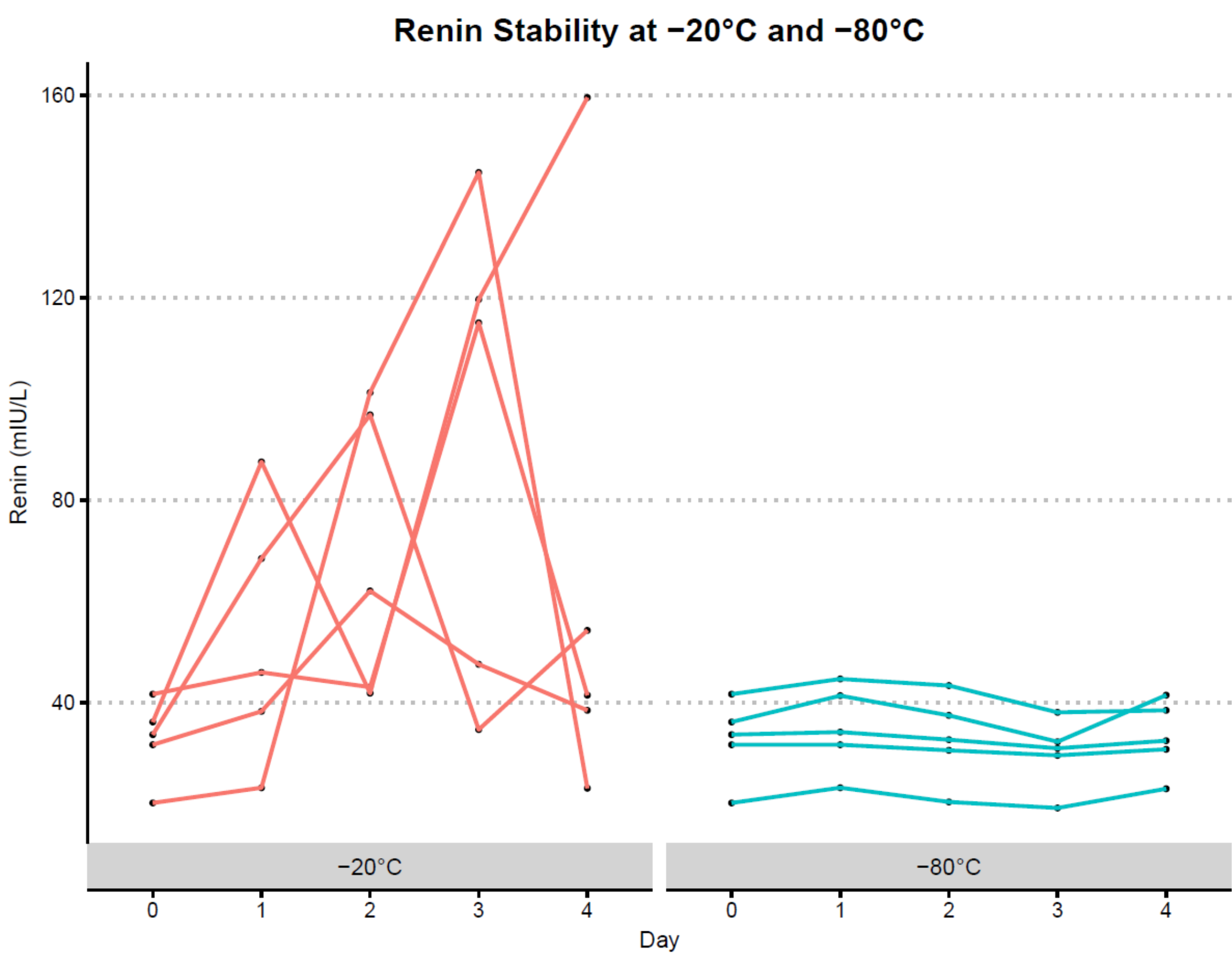


Figure 2: Renin concentrations from volunteers over five days, under the two storage conditions.

Conclusions

Storage of plasma renin samples at -20°C does not prevent cryoactivation, which is highly variable. Cryoactivation is most significant for patients being screened for primary hyperaldosteronism, as it may result in a falsely low ARR. Sample handling at room temperature is recommended and otherwise with storage at -80°C where appropriate. We have now instigated storage at -80°C for our renin samples in light of these findings.

Acknowledgments

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References

- Rutledge AC et al. Survey of renin and aldosterone testing practices by Ontario laboratories – Providing insight into best practices. Practical Laboratory Medicine. 2021;25:e00229.
- Özcan Ö, Hillebrand JJ, Elzen WD, Heijboer AC. The clinical impact of sample storage at -20°C on renin reference intervals and aldosterone-renin ratio calculations. JCEM. 2024;109(6): 1472-1475.
- Bonnitcha P, Rigdwell M, Ward P, Chesher D. Standard -20 °C freezer storage protocols may cause substantial plasma renin cryoactivation. CCLM. 2023;61(8):1428–1435.