

## ORIGINAL ARTICLE

# Impact of Storage Conditions on the Stability of Urinary Biomarkers

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## SUMMARY

**Background:** This study aimed to assess the influence of different storage conditions and pH levels on biomarkers in urine.

**Methods:** Urine samples were collected from 20 healthy individuals and 20 patients with kidney disease. The stability of nine proteins with normal and pathological concentrations was investigated under 4 types of storage conditions: 25°C for 8 hours, 4°C for 7 days, -20°C for 12 weeks, and -80°C for 12 months. Ten pH values (4.0 to 8.5) were also tested. Nine biomarkers included U-α1MG, U-Alb, U-Trf, U-IgG, U-NAG, U-NGAL, U-RBP, U-CysC, and U-TP, mostly measured in the laboratory.

**Results:** U-Alb, U-α1MG, U-Trf, U-TP, and U-CysC were relatively stable across four storage conditions. However, the stability of other biomarkers may be impacted by the storage conditions. For instance, U-IgG was unstable at -20°C after eight weeks and -80°C after one month. U-NAG was unstable at 4°C after four days and -20°C for 12 weeks. U-RBP was unstable at -80°C after five months and showed a significant upward trend at -20°C within 12 weeks in the pathological levels. Regarding the impact of the pH levels, the bias of U-α1MG and U-CysC did not exceed ± 10% across the pH range of 5.0 to 7.5. The bias of another 5 biomarkers (U-Alb, U-Trf, U-IgG, U-NGAL, and U-TP) was less than -10% at pH 5.0 to 6.0. U-NAG and U-RBP were unstable, with bias exceeding -10%.

**Conclusions:** U-NAG and U-RBP should not be stored at -20°C. U-IgG could only be stable for a short period under -20°C and -80°C. Urinary pH should be monitored and adjusted if necessary.

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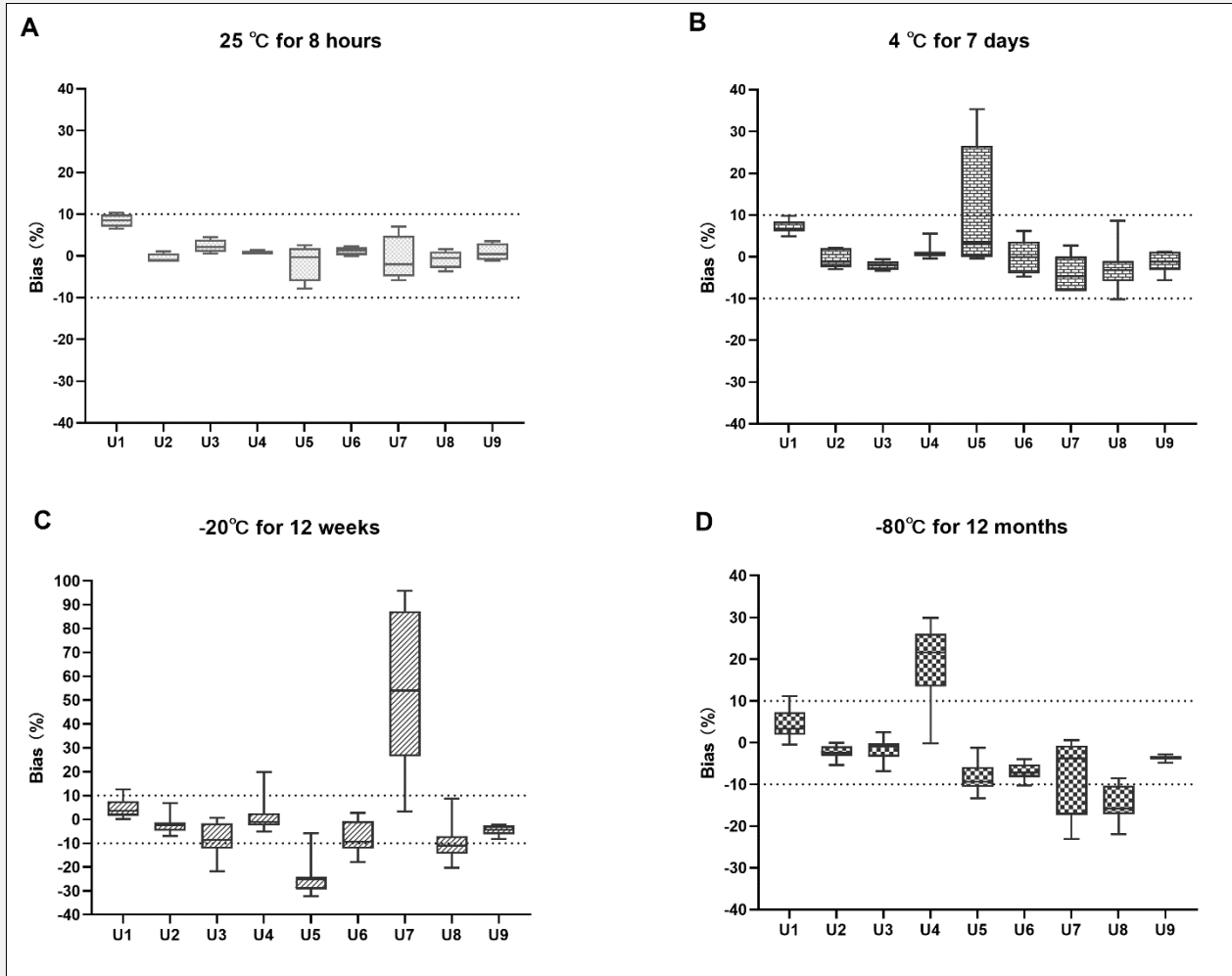
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# Supplementary Data



**Figure S1. The bias of nine biomarkers in the pathological urine levels under four storage conditions.**

Numbers 1 - 9 represent U- $\alpha$ 1MG, U-Alb, U-Trf, U-IgG, U-NAG, U-NGAL, U-RBP, U-CysC, and U-TP. The dashed line shows a variation of  $\pm 10\%$ .

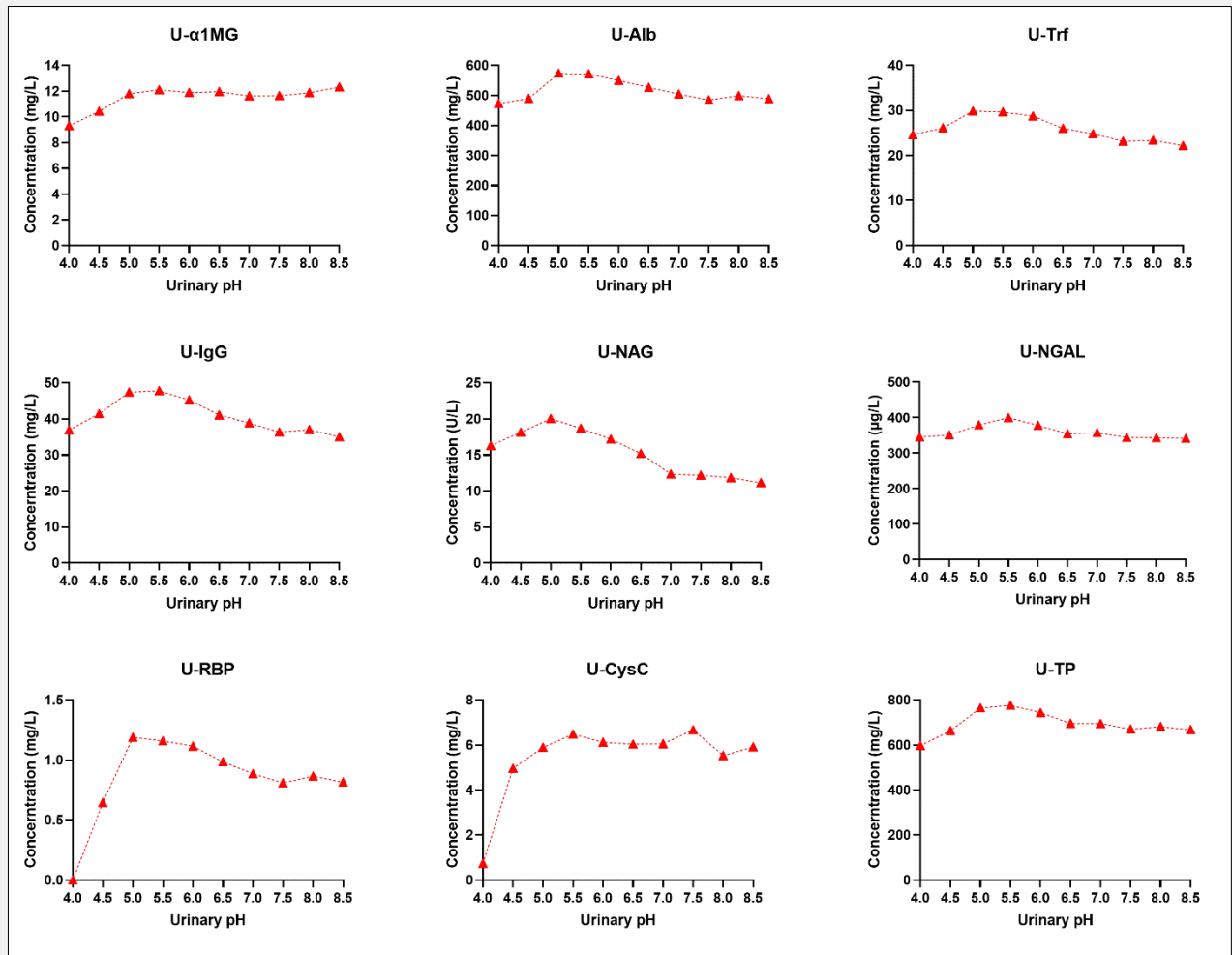


Figure S2. The concentrations of nine biomarkers in pathological-level urine across different pH levels.