Sir,

Albuminuria is used as a predictor of events in many epidemiological studies and as an intermediate end-point in clinical trials. We have recently shown that frozen storage results in assessment of falsely low urinary albumin concentrations (UAC), particularly in the reference and microalbuminuric range (UAC 0–200 mg/l) [1]. If samples were subjected to hand inversions or vortex-mixing prior to assessment, UAC remained almost unchanged (2.6–5.3% decrease) after 7 days of storage, whereas the difference increased to ~25% if samples were stored for 18–24 months.

It has been suggested that urinary pH is a determinant of the decline associated with freezing, but to the best of our knowledge, there is only one study that has actually investigated this issue. In this study, Erman et al. [2] found no association between urinary pH and changes in UAC after 7 days of storage at −20°C. We investigated whether urinary pH is a determinant of changes in UAC after storage at −20°C for 4 and 12 months.

Urine samples (UAC > 5 and UAC < 200 mg/l, n = 230) were collected by participants during the third screening (2004–05) for the Prevention of REnal and Vascular ENd-stage disease (PREVEND) study (www.PREVEND.org). pH was measured by Combur strips (pH range 5–9, expressed as integers), UAC was assessed by immunonephelometry (Dade Behring, Germany) in fresh urine samples and after frozen storage in polypropylene aliquots. Samples were subjected to hand inversions and centrifugation prior to assessment.

Median UAC (interquartile range) was 10.7(7.3; 51.3) mg/l in fresh samples at baseline. Urinary pH was 5 in 38%, 6 in 44%, 7 in 15% and 8 in 3% of cases. After 4 months of storage, the change in UAC was −29(−59; −8)% at pH 5, −20(−56; 2)% at pH 6, −10(−48; 2)% at pH 7 and −6(−38; −5)% at pH 8 (P < 0.001 for trend). The results for 12 months of storage are shown in Figure 1. We conclude that urinary pH affects the magnitude of decline in UAC after prolonged frozen storage. The mechanism underlying this observation may involve increased aggregation and denaturation of albumin at relatively low pH, because of the isoelectric point of albumin at pH 4.7. One study has been performed in which urinary pH was adjusted before storage at −20°C [3]. In this study, pH was adjusted to 7, and storage was for 8 weeks. Results were nevertheless promising: there was no significant decrease after adjustment of pH, while UAC decreased significantly in unadjusted samples. Adjustment of pH may be a feasible option for better preservation of urine samples that are meant to be stored for a prolonged period before assessment of albumin.

Conflict of interest statement. None declared.

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Fig. 1. Change in UAC after 12 months of storage at −20°C according to pH by boxes-and-whiskers plot. The boxes indicate lower and upper quartiles and the central lines are medians. The whiskers represent 95% confidence intervals.

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