Quantitative Determination of Low and High Molecular Weight Proteins in Human Urine: Influence of Temperature and Storage Time, Ina S. Klasen,1* Louis J.M. Rechert,2 Corrie M. de Kat Angelino,1 and Jack F.M. Wetzels2 (Departments of 1 Clinical Chemistry and 2 Nephrology, University Hospital St. Radboud, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands; * author for correspondence: fax 0031-243541743, e-mail I.Klasen@CKCL.AZN.NL)

Many authors have studied the influence of storage on measured albumin concentrations in urine. Whereas some authors report that albumin concentrations decrease upon storage of urine at −20 °C (1–10), others cannot confirm this (11–14). As has been discussed extensively in the literature (15), these seemingly discrepant results might be explained by the use of pooled urine vs individual urine samples. The effect of storage may differ between various urine samples, probably because of variables such as protein concentrations, pH, and ionic strength. As a consequence, pooling of the urine samples might dilute the storage effect that can be found when using individual urine specimens. Other variables that have been tested for their influence on the measurements of urinary proteins are pH (1–4, 13, 16–18), the tube material (4, 19, 20), handling of the sample (1, 7, 10, 12, 14), and the use of additives (21–23).

It is likely that proteins other than albumin are also influenced by storage conditions, but information about the extent of such effects is limited (1, 3, 16–18, 21–23). In the study presented here, we measured several urinary proteins by nephelometry [albumin, transferrin, IgG, and α1-microglobulin (α1-m)] or by an RIA [β2-microglobulin (β2-m)] in urine samples stored at different temperatures over prolonged time periods.

Spot urine samples were collected from 15 patients attending the outpatient nephrology clinic (after obtaining informed consent of the patient). The ranges of the protein concentrations measured in the fresh urine were as follows: albumin, 23–17670 mg/L; transferrin, 2.2–1120 mg/L; IgG, 3.8–887 mg/L; α1-m, 3.9–379 mg/L; and β2-m, 0.1–37.3 mg/L. After centrifugation (1500g for 15 min), the supernate was divided into aliquots of 2 mL in polystyrene tubes. At least one tube was used for each storage condition, to avoid freezing and thawing. The urine samples were stored at three temperatures (4, −20, and −70 °C) with and without addition of 30 g/L bovine serum albumin (BSA; Sigma Chemical Co.). No BSA was added to samples in which albumin was to be determined, because of cross-reactivity. Protein concentrations were subsequently measured at 1, 2 and 4 weeks (4 °C) or at 1, 4, 12, 26, and 104 weeks (−20 and −70 °C). After being thawed, all urine samples were mixed by inversion and vortex-mixing and again centrifuged (1500g for 15 min). Transferrin could not be detected in the urine of one patient. In five urine samples, β2-m could not be measured because of a low pH (n = 4) or technical problems (n = 1).

Albumin, transferrin, IgG, and α1-m were measured by nephelometry. The apparatus, calibrators, controls, and diluent were as described previously (5). For all four proteins, six-point calibration curves were used.

β2-m was measured by an RIA according to instructions of the manufacturer (Pharmacia). β2-m was not measured when the pH of the urine was below 6.0, because the protein is unstable at low pH values (16).

The interassay CVs of all methods were ~6%. The main results of the study are presented in Fig. 1. The effect of each tested variable is discussed below.

At −20 °C, the protein concentrations in several urine samples decreased with time, whereas results obtained for urine samples stored for 2 years at −70 °C were quite acceptable (Fig. 1). Storage for no more than 4 weeks at 4 °C was the most reliable (data not shown).

Because urine samples for research purposes are sometimes stored for many years, it might be convenient to freeze them at −70 °C and store them at higher temperatures later. Therefore, after freezing urine samples at −70 °C we transferred some urines to −40 °C. Unfortunately, the protein concentrations of these urines were also unstable (data not shown). This indicates that not only is the initial freezing process essential for the preservation of the proteins, but that the long-term storage temperature is also important.

Not all urinary proteins were similarly sensitive to storage, nor did they react in the same manner. A substantial decrease (>20%) occurred most often for IgG, and to a lesser extent for transferrin, α1-m, and β2-m. Increases in protein concentrations, however, were sometimes found for transferrin and α1-m. Urinary albumin was relatively stable at the storage conditions tested.

The effects of storage on the measurement of the urinary proteins were not uniform for all urine samples. We did not find any relationship between characteristics such as pH, osmolality, creatinine, or initial protein concentrations and the extent of changes in protein concentrations. However, we noticed that protein concentrations tended to be lower particularly in samples that formed precipitates after storage and thawing. We do not know whether these lower protein concentrations are the result of protein loss, or whether the proteins are immunologically changed and therefore no longer measurable in our assays.

The change in urinary protein concentrations over time differed for each urine sample and each protein. As seen in Fig. 1, it is clear that after storage for only 1 week at −20 °C IgG concentrations decreased. This effect became even more pronounced over time. Comparable results, with somewhat smaller decreases, were obtained for transferrin, α1-m, and β2-m.

In several studies additives were used (21–24). We have used BSA to prevent adhesion of proteins to the tubes. BSA, however, did not prevent the decrease of protein concentrations at a storage temperature of −20 °C (data not shown). Tencer et al. (22, 23) have reported the beneficial effect of the addition of a preservative solution on the recovery of urinary proteins stored at −20 °C. This may be one option to overcome instability of urinary
Fig. 1. Protein concentrations expressed as percentages of the initial values of 15 urine samples measured at various time intervals after storage at -20 or -70 °C. The baseline α1-m concentration of the urine sample that showed an increase was 10.8 mg/L.
proteins. We show here that storage at −70 °C is another option.

Urinary concentrations of albumin were remarkably stable in this study, which seems somewhat in contrast to our previous work (5) and to the findings of other investigators (1–4, 6–10). We feel that this discrepancy can be explained by the fact that most studies involved the measurements of albumin in the microalbuminuric range, whereas in the present study most urine samples contained high concentrations of albumin. Therefore, our conclusion can be applied only to urine samples that contain relatively large amounts of albumin. In our study, IgG was more sensitive to storage effects than albumin, and we would expect that losses of IgG might be even more pronounced in urine samples with low protein concentrations. Recent data presented by Hoogenberg et al. (24) seem to confirm this idea. The use of BSA may be warranted when urinary protein concentrations are low (except for albumin measurement because of cross-reactivity).

We conclude that if urinary proteins can be measured within 4 weeks after sampling, storage at 4 °C appears to give the best results. For longer time periods, a storage temperature of −70 °C should be chosen. Storage at −20 °C (without appropriate preservatives) is not recommended.

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References

Evaluation of the New AxSYM Cyclosporine Assay: Comparison with TDx Monoclonal Whole Blood and Emit Cyclosporine Assays, Pierre E. Wallenmacq* and Kathy Alexandre (1 Laboratory of Therapeutic Drug Monitoring, Department of Clinical Chemistry, University Hospital St Luc, University of Louvain, 10 Hippocrate Avenue, B-1200 Brussels, Belgium, and 2 Abbott Diagnostics Division, Rue du Bosquet 2, B-1348 Ottignies/Louvain-la-Neuve, Belgium; * author for correspondence: fax 32 2 764 90 44, e-mail wallenmacq@lbcm.ucl.ac.be)

Cyclosporine (CsA) is a cyclic undecapeptide that has potent immunosuppressive activity, but its narrow therapeutic range and variable pharmacokinetics in humans make monitoring of CsA mandatory (1). The generally accepted whole-blood therapeutic ranges of CsA (with specific assay) are 100–200 µg/L for renal transplant patients and 150–250 µg/L for cardiac, hepatic, and pancreatic transplant patients during the maintenance phase (2,3), whereas slightly higher ranges are recommended during the induction phase. The CsA metabolites arising from oxidative pathways (4) cross-react in immunoassays (5,6), leading to results higher than those by HPLC. Trough concentrations of metabolite AM1, the major metabolite in human blood (1,4), can exceed those of the parent drug (1). For metabolites AM1, AM9, and AM4N, immunosuppressive activity is <10% of that observed for CsA (7,8), and at least in rats, various CsA metabolites are not nephrotoxic (9). Thus CsA itself,