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Short communication

Effects of Storage Temperature, pH and Time on Urinary Albumin Level

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ABSTRACT: The storage of urine samples at 2-8^{o}C and at -20^{o}C for several weeks is a common laboratory practice in research and epidemiological studies to facilitate batch analysis. Despite numerous studies, there is no agreement on how stable albumin is in urine and the best way to store such urine specimen. Random urine samples were obtained from 15 patients with nephropathy and end stage renal failure. Urinary pH and specific gravity of freshly voided urine were determined before storage. A portion of each urine samples were stored at voided pH and another portion stored at neutral pH (7.0) after adjustment with acid or base at 4^{0}C and -20^{0}C for 10 weeks. Pre -analytical treatment of samples involved vortex mixing or centrifuging urine samples before measurement of urinary albumin. Albumin levels were determined at 0, 2, 4 and 10 weeks in both vortex mixed and centrifuged samples using standard spectrophotometric methods. Mean pH and specific gravity of fresh urine samples were 5.82 ± 0.71 and 1.009±0.004 respectively. Significant decreases ($p< 0.05$) were observed in urinary albumin levels at 4^{0}C at the 4th week. Significant changes were seen at the 10th week in samples stored at -20^{0}C in pH unadjusted samples. Vortex mixing or centrifuging of sample of did not restore decreases in albumin level. No significant difference was observed in the pH adjusted group after 10 weeks of storage at -20^{o}C.These results suggest that where long term storage (6-10 weeks) of urine samples is required, samples should be stored at -20^{0}C. However, in medium term storage (2-4 weeks) storage at 4^{0}C may require the adjustment of the pH to neutral (7.0) before storage to obtain reliable results.

Keywords: Urinary albumin, temperature, pH, time, storage

INTRODUCTION

Estimation of total protein content of urine is regarded as a useful test in the diagnosis and management of diseases especially renal diseases. Factors such as temperature, pH, and the ionic strength of a solution affect the stability of proteins in solution (Ronald *et al*, 1984) while type of diet could affect urine pH (Taylor *et al*, 1978)

The system of changing the pH of urine sample before storage show that inappropriate pH can invalidate results of albumin estimation while proteinuria elevates specific gravity value (Ronald *et al*, 1984). Urinary albumin level was stable for 2 days at room temperature and 2 weeks – 6 months at –20^{0}C (Gatling *et al*, 1988). However, losses of 5% per year occurred for as long as 5 years (Gianpetro *et al*, 1991). So far, the issue of specimen storage remains largely unsettled and demand further investigations. The study was aimed at finding acceptable conditions for optimum preservation of urinary albumin by adjusting the pH of urine samples before storage.

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MATERIALS AND METHODS

Random urine specimens, obtained from 15 patients with nephropathy and end stage renal failure admitted in the Nephrology Unit of the University College Hospital, Ibadan were used for the study. Pre-analytical treatment of samples was carried out as shown below in table 1.

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Table 1:
Types of Pre-analytical Treatments of Urine Samples

Types of Treatment	Aliquot 1	Aliquot 2
Stored at voided pH	Yes	_
pH adjusted to neutral (7.0) before storage	_	Yes
Sample centrifuged at 3000rpm X 5 minutes before assay	yes	Yes
Sample vortex -mixed before assay	yes	Yes

Urine samples were stored at 4^0C and -20^0C for 10 weeks. Urinary albumin levels were determined by the spectrophotometric dye (bromophenol blue) binding method of Schosinsky *et al*. (1987) at baseline, 2, 4, and 10weeks.

The pH adjustment of freshly voided urine samples to neutral (7.0) was carried out using 1.0M sodium hydroxide (NaOH) for urine samples with pH <7.0, while 1.0M hydrochloric acid (HCl) was used for urine samples with pH >7.0. The specific gravity of fresh urine samples was determined using a urinometer (PSL Cat no.916)

RESULTS

The mean ± SD of the specific gravity (SG) and pH of the fresh urine samples were 1.0009 ±0.004 and 5.8± 0.7 respectively while the baseline value of albumin in urine was 4.74± 2.4. As shown in table 2, a decrease in the mean urinary albumin level was observed throughout the period of storage at 4^0C. The observed decrease persisted irrespective of the type of pre-analytical treatment of sample.

There were no significant changes in mean albumin values after 2 weeks of storage at 4^0C when compared with corresponding baseline value in all the treatment groups. However, significant changes were observed after 4weeks in samples whose pH was not adjusted to neutral before storage.

Vortex mixing of samples before assay improved the result in the neutral pH group hence there was no statistically significant difference in the mean albumin value when compared with the corresponding baseline. However, assay of the clear supernatant after centrifugation at 3000rpm x 5minutes did not improve the outcome.

For samples stored at -20^0C, a general but gradual decrease in the mean albumin value throughout the period of storage as seen in table 3. No significant changes were observed after 2 and 4 weeks of storage. After 10 weeks, significant decreases were seen in samples stored at voided pH. Vortex mixing or centrifuging sample did not improve the outcome. In contrast, urine samples stored at neutral pH remained fairly stable and no significant differences in mean albumin value were observed when they were compared with the corresponding baseline value.

Table 2:
Mean ± SD (g/L) of urinary albumin and p- values of all treatment groups stored at 4^0C for 2 and 4 weeks.

Type Of Treatment	**Baseline**	**2 weeks**	**p- value**	**4 weeks**	**p- value**
Stored at pH 7.0	4.7±2.3				
Vortex Mixed		4.4±1.5	ns	3.6± 1.8	ns
Centrifuged		4.2± 2.2	ns	3.2±1.6	<0.05
Stored At Voided pH	4.7±2.3				
Vortex Mixed		4.2±2.1	ns	3.3±1.6	<0.05
Centrifuged		4.2±2.1	ns	3.9±1.6	<0.05

Table 3:
Mean ± SD (g/L) of urinary albumin and p- values of all treatment groups stored at -20^0C for 2, 4 and 10 weeks.

Type of treatment	**Baseline**	**2 Weeks**	**p-Value**	**4 Weeks**	**p- Value**	**10 Weeks**	**p-Value**
Stored At pH 7.0	4.7 ± 2.3						
Vortex Mixed		4.6 ±2.3	ns	4.6±2.3	ns	4.1±2.0	ns
Centrifuged		4.5 ±2.3	ns	4.4± 2.3	ns	3.9±2.1	ns
Stored At Voided pH	4.7 ± 2.3						
Vortex Mixed		4.5 ± 2.3	ns	4.3± 2.2	ns	3.2±1.7	<0.05
Centrifuged		4.5±2.0	ns	4.1±2.1	ns	2.9±1.7	<0,0.

DISCUSSION

The stability of proteins in solution is dependent on the structure and the ionic strength of the solution. Extreme ionic strength can denature or destroy protein structures. Denatured proteins are generally less soluble and often precipitate in solution. The formation of precipitates may lead to the underestimation of proteins such as albumin. In this study, significant decreases ($p<0.05$) in the urinary albumin levels were observed in samples stored at 4^0C after 4 weeks but not at the 2nd week in urine samples whose pH was not adjusted to neutral before storage. Physical examination of these samples revealed the presence of precipitates in samples only after 4 weeks. However, particulate matter was seen in all the samples. Vortex mixing or centrifuging the samples at 3000rpm x 5 minutes did not seem to affect the outcome of the results. Presumably, albumin may be trapped in these precipitates leading to a significant decrease in the results. These observations are similar to those of Elving and colleagues[6] but in contrast with that of Hara *et al* (Hara *et al*, 1994).

The results obtained from the assay of urine samples stored at -20^0C were similar to those stored at 4^0C at the second week. Thereafter, significant differences were seen both at the 4th and 10th week.

No significant difference was seen in the urine samples in all treatment groups after 4 weeks of storage at -20^0C. However, at the 10th week, significant changes were observed in those samples whose pH was not adjusted to neutral before storage. Our finding is in agreement with a previous observation by Schultz *et al* (2000) who noted that storage of urine samples at -20^0C led to a variable underestimation of urinary albumin in 28% of specimen examined. In contrast however, Collins and coworkers (1993) examined the stability of albumin in urine under different storage conditions and concluded that there was no significant difference in urinary albumin concentration between fresh urine samples and during 6 weeks of storage at -20^0C. This observed difference might have resulted from the time of storage since our study lasted for 10 weeks, while theirs was for 6weeks.

No significant differences were observed in those samples whose pH was adjusted to neutral before storage after 10 weeks at -20^0C. The stability might be due to the inclusion of particulate matter in the assay because when same samples were centrifuged at 3000 rpm X 5 minutes and the clear supernatant assayed, statistically significant differences were observed.

Our observations support previous findings that storage of urine sample for the estimation of albuminuria results in a partial albumin and protein sedimentation and may lead to the underestimation of the real values to varying degrees. Doumas and Peters (1997) suggested however that the safest way is to analyze urine as soon as possible or if analysis is delayed for a few days, keep them in a refrigerator.

Our findings on the other hand suggest that where long term (6-10weeks) storage is desired, urine samples should be stored at -20^0C with pH adjusted to neutral before freezing. Vortex mixing of samples to include particulate matter before assay tends to produce more reliable results than the assay of the clear supernatant.

REFERENCES

Collins A.C., Seith M., MacDonald F.A., Brown D., Viberti G.C. (1993): Storage temperature and differing methods of sample preparation in the measurement of urinary albumin. Diabetologia 36:993-997.

Doumas B.T. and Peters Jr. T. (1997): Serum and urine albumin: a progress report on their measurement and clinical significance Clin Chim Acta 258: 3-20.

Elving L.D, Bekkeren J.A, Janson M.J., Angelino C.M., de Nobel E., van Munster P.J. (1989): Screening for microalbuminuria in patients with diabetes mellitus: frozen storage of urine samples decreases their albumin content. Clin Chem. 35: 308-310.

Gatling W., Knight C., Mulee M. A. Hill R. D. (1988): Microalbuminuria in diabetes: a population study of the prevalence and assessment of three screening tests. Diabetic Med 5:437 –440.

Gianpetro O., Clerico A., Cruschelli L., Penno G., Navalesi R. (1991): More on effect of storage time and temperature on measurement of small concentrations of albumin in urine Clin Chem 37:591-592.

Hara F., Nakazato K., Shiba K., Shimoda J., Kojima T., Fukumura Y., Kbayishi I. (1994): Studies of diabetic nephropathy I: Effects of storage time and temperature on microalbuminuria. Biol. Pharm Bull. 17:1241-1245.

Ronald H. NG., Menom M., Jack H. I. (1984): Collection and handling of 24 hour urine specimen for measurement of analyte related to renal calculi Clin. Chem. 30: 461-471.

Schosinsky K.H., Vargas M., Esquivel A. L., Chararria C.A. (1987): Simple spectrophotometric determination of urinary albumin by dye binding with use bromophenol blue. Clin Chem 32: 223-226.

Schultz C. J., Dalton R.N., Turner C., Neil H.A., Dunger D.B. (2000): Freezing method affects the concentration and variability of urine proteins and the interpretation of data on microalbuminuria. The Oxford Regional Prospective Study Group Diabet Med. 17:7-14.

Taylor G.O., Oyediran A.B.O., Adesina H.A. (1978): The effect of seasonal changes and socioeconomic status on urinary pH and specific gravity. Trop. Geog. Med. 31: 105-110.