

Influence of the storage temperature on urine analysis in timed samples

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Introduction

The collection of a urine specimen over a predetermined time interval has the advantage of minimizing the short term biological variation of analytes. It is important that the patient follows close instructions for urine collection to obtain a proper 24-hours urine sample (1). One essential pre-requisite is that the collected urine should be kept in the refrigerator. In common practice, however, the storage of urine under cold temperatures seems unpractical and is often uncared for. When collected over the weekend, samples might be sent to the laboratory for analysis 24-72 hours after collection. To circumvent the problem of failure to refrigerate the sample when stored for longer periods, laboratories add preservatives before collection to reduce bacterial action or chemical decomposition. A disadvantage of the use of preservatives, however, is the possibility of interference with analytical methods. We investigated the effect of storage temperature and bacterial growth on the concentration of a panel of analytes.

Methods

Sample handling

Nitrite-positive urine was collected from three different patients. The presence of bacteria was confirmed using microscopy. All specimens were divided into aliquots and stored at either at room temperature (RT) or at 4 °C in the presence or absence of 0.15 mmol/l sodium azide (NaN₃, Merck, 26628-22-8) to prevent further bacterial growth.

Methods of analysis

Urinalysis was performed after 0, 24, 48 and 72 hours of storage. Urine samples were centrifuged, the supernatant was collected and was analysed on a ADVIA® 1650 Chemistry system (Siemens) for albumin (pyrogallol red-molybdate complex formation), amylase (ethylidene blocked pNPG7 enzymatic method), protein (biuret complex formation), creatinine (picric acid kinetic method), potassium, sodium (indirect ISE) and urea (enzymatic reaction utilizing urease and glutamate dehydrogenase). Bacterial growth was monitored by manual counting using light microscopy.

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Statistics

Analyte concentrations/activities from different time points were compared with their respective concentration measured at time point zero. Relative changes in concentration/activity after incubation to concentration at time point zero were calculated for different storage times. Results from urine collected from three different patients qualitatively positive for the presence of bacteria were pooled for all different time points and the mean +/- standard error of the mean between storage at RT or at 4°C was calculated. Statistical analysis was performed using a Wilcoxin test.

Results

The effect of storage conditions on analyte stability was investigated by comparing the urine analyte concentration after storage at room temperature (RT) and at 4°C for various time intervals (from 24 hours up to 72 hours). Almost all analyte concentrations slightly changed due to storage regardless the storage temperature (table 1). In most cases these changes did not exceed the analytical precisions. In contrast, the analyte stability of protein, potassium and amylase were markedly affected by storage temperature. While the

Table 1. Preanalytical variation introduced by storage of timed urine samples. Urine containing bacteria was collected and stored either at RT or at 4 °C. At time point zero, after 24, 48 and 72 hours urine samples were analyzed for sodium, potassium, urea, amylase, albumin, protein, creatinine and urate. Results are expressed as mean percentage deviation between concentration/activity of the analytes at time point 24 hours, 48 hours or 72 hours from concentration/activity of the indicated analytes at time point zero. The maximum allowable bias is indicated for 24 hour timed urine sample (2), except ¹first morning urine sample and ¹¹random urine sample.

	Mean percentage deviation from concentration at t=0 (% ± SEM)		p-value	Analytical precision (%)	Max. allowable bias (%)
	4°C	RT			
Na ⁺	0.0 ± 0.3	0.4 ± 0.5	>0.05	1.0	9.0
K ⁺	1.6 ± 0.7	4.2 ± 1.0	0.006	1.5	8.9
Urea	0.1 ± 1.6	0.3 ± 1.7	>0.05	2.6	8.6
Amylase	-0.8 ± 0.5	3.3 ± 0.8	0.016	3.3	26.2 ⁽¹⁾
Albumin	2.5 ± 1.6	2.2 ± 2.6	>0.05	5.8	16.4 ⁽¹¹⁾
Protein	0.0 ± 3.1	49 ± 14	0.016	4.2	10.9
Creatinine	2.1 ± 0.5	1.7 ± 0.7	>0.05	2.4	8.6
Urate	-5.5 ± 1.6	-2.0 ± 1.4	>0.05	6.1	8.3

concentration protein, potassium and amylase activity significantly increased after storage at RT compared to the initial concentration/activity in the urine sample, no such increase in concentration/activity was observed after incubation of the urine specimen at 4 °C. The relevance of the increases of the latter two analytes may be limited since the observed bias does not exceed the maximal allowable bias for potassium and amylase (2). Total protein concentration was increased on average with 49% when stored at RT compared to time point zero, which could be prevented by storage at 4 °C. The increase of 49% exceeded the maximal allowable bias, indicating that storage of urine specimens at RT contributes to both significant and relevant positive bias in protein concentration. The increased protein concentration cannot be explained by an elevation in albumin, since albumin concentration was similar in urine specimens stored at RT and 4 °C. Whether other methods of analysis of urinary protein are of influence remains to be determined.

Taken together, the influence of storage conditions of urine specimens on analyte concentration varies with the substance tested. Prolonged storage of urine at RT introduced a significant positive bias in potassium, protein concentration and amylase activity, while sodium, urea, albumin, creatinine and uric acid con-

centration is stable for up to 72 hours irrespective of storage temperature. To investigate whether the propagation of bacterial growth at RT could contribute to the differences observed between storage at RT and at 4 °C, analyte stability was monitored in the presence or absence of bactericidal levels of NaN_3 . Indeed, bacterial growth was prevented by incubation of the urine sample at 4 °C, while storage at RT propagated bacterial growth (figure 1). Addition of 0.15 mmol/l NaN_3 could clearly prevent propagation of bacterial growth at RT. Analysis of potassium and protein concentration showed similar increases in concentration irrespective of the presence of NaN_3 . Thus, bacterial growth alone could not account for the observed bias introduced in potassium and protein concentration by storage at RT, since prevention of bacterial growth does not diminish this bias.

Conclusion

This report describes the experimental evaluation of storage temperature and bacterial growth on analyte stability. We find no significant differences between storage at RT or 4 °C for up to 72 hours in the mean analyte concentration of sodium, urea, albumin, creatinine and uric acid, indicating the stability of these analytes after prolonged storage irrespective of the storage temperature. Although recommended to curtail bacterial growth in non-sterile urine samples, these results suggest that cold storage of timed urine samples is unnecessary. This would eliminate the inconvenience for patients to refrigerate 24-hour urine samples. The notable exceptions to the observed independence of storage temperature are protein and potassium concentration and amylase activity, in which pre-analytical errors were introduced by storage at RT. Especially the protein content in timed urine samples is critically dependent on storage of urine at 4 °C. Initial experiments showed that the increase of protein concentration at RT conditions could not be explained merely by bacterial growth. In light of these results, refrigeration of urine samples is a prerequisite for the avoidance of pre-analytical errors in protein and potassium concentration and amylase activity. Overall, the unavoidable improper storage at RT of 24 hour urine by the patient does not introduce significant pre-analytical errors in most analytes. However, the pre-analytical bias introduced in potassium and protein concentration by storage at RT should be taken into account in urinalysis of timed urine samples.

References

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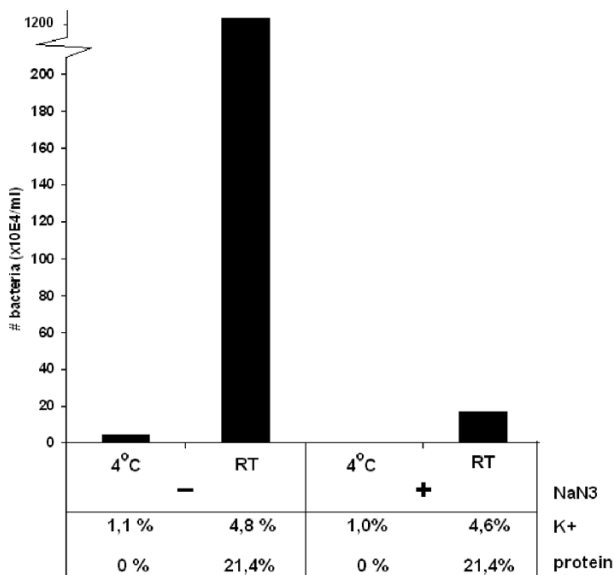


Figure 1. Inhibition of bacterial growth in urine does not prevent temperature-dependent positive bias in protein and potassium concentration. Urine containing bacteria was collected and stored at RT or at 4°C in the presence or absence of 0.15 mmol/l NaN_3 to prevent further bacterial growth. The indicated amount of bacteria in urine samples was determined after 72 hours. At time point zero, after 24 and 72 hours urine samples were analyzed for potassium and protein concentration. Results are expressed as mean percentage deviation between concentration of the analytes at time point 24, 48 or 72 hours from concentration of the indicated analytes at time point zero.