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Original Research Article

Effect of storage duration and analyte concentration on measurement of neutral pH clinical chemistry urine analytes

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ABSTRACT

Background: The aim of the study was focused very keenly in measuring the changes in concentration of sodium, potassium and creatinine in urine, stored for 60 days effect of urinary storage duration of clinical chemistry analytes. This study determined whether patient urine samples can be used for day to day urine quality control.
Methods: Spot urine samples were collected from left over samples from the admitted patients in various hospital wards. The samples are kept which its pH was unaltered or unspiked and was aliquoted and deep fridged immediately. These aliquots were melted at an incubator temperature of 86 ºC and was analyzed in the analyzer for 60 days and data were collected for checking variations.
Results: With the strenous and continious monitoring from the side of the researchers, the researchers had throughly analysed and found that in both the sets almost all of those analysed urine test of pH 5 and analytes remained very much stable for a period of 60 days which could be used for running daily internal quality controls.
Conclusions: Patient urine can be used as internal quality control sample for at least 60 days for sodium, potassium and creatinine. During the 60 days period, there is no appreciable degradation of any of the two sets of samples for sodium, potassium and creatinine measurements.

Keywords: pH, Sodium, potassium and creatinine standard deviation, Coefficient of variation, Quality control

INTRODUCTION

Sodium, potassium and creatinine as neutral pH analytes are requested occasionally by clinicians in urine for several clinical decisions making. Although, its request is far more common than that of requests for calcium, phosphorus and uric acid in serum, nevertheless their internal and external quality control is essential to give clinical reliable results. Due to their less common request from clinicians, procuring their internal quality control material is a financially un-viable option for most small and medium sized laboratories, more so in publicly funded laboratories in government health services. Use of patient samples for internal quality control is an alternative to commercial IQC material. Such samples must be preserved in such a way that samples and analytes measured do not deteriorate in any way for a reasonably long time for use in an IQC program as an alternative to commercial IQC material. Moreover many commercial IQC materials have lower analyte range than found in many patients' urine. While many analytes in urine are supersaturated in physiological fresh urine, it is difficult to keep it supersaturated in preserved urine for long without altering its storage pH. This study measured stability of various analytes at various pH at usually found concentration as well as at abnormally high concentration of analytes. Kidneys glomeruli freely filtered urinary sodium. Kidneys glomeruli freely filters urinary sodium. 80% of the sodium is reabsorbed in proximal tubules and 20% in the loop of Henle. Urine factors play a role in kidney stone formation as in 24 hour excretion of calcium, oxalate, uric acid, a total urine

volume, sodium, phosphorus and urine pH has been reported to stone risk formations.[1] The urinary buffer mechanisms decreases the preferentially transported species, has a direct effect of pH on proximal tubule apical phosphate transport.[2] Acute tubular necrosis occurs as the internal renal failure secondary to ischemic. Histopathologic findings of ATN are limited to the tubulo-interstitium and often subtle despite profound dysfunction.[3] Thiazides are far less expensive than the newer antihypertensive agents and are favored in terms of cost minimization.[4] Prerenal azotemia is increased in serum creatinine and BUN and also with oliguria due to profused tubular reabsorption of sodium. QC systems are most sensitive to analytical errors around the decision limit.[5] 80% of the sodium is reabsorbed in proximal tubules and 20% in the loops of Henle. Urine factors play a role in kidney stone formation. A 24 hour excretion of a total urine volume, sodium, potassium, creatinine with other mineral and urine pH has been reported to stone risk formations. Na+, K+ ATPase activity is higher in the distal convoluted tubule. Distal convoluted tubules have the main function of electrolyte reabsorption. ANP hormone situated on the renal epithelial cells acts by ionic channels. Analytical procedures for sodium and other calcium, phosphorus, uric acid salts along with creatinine in 24 hour urine collections is often needed for the differential diagnosis of patients with renal calculi.[6] The diagnostic goal is based on assessing the volume status, to establish the renal hypoperfusion and to determine the causative etiology.[7] Electrolyte abnormalities are usually corrected by changing the formulations, flow rates of replacement, dialysate solution or by supplementation.[8] For smooth diagnosis, the therapy can be initiated promptly. Seasonal variation in acute kidney injury has been associated with variation of AKI incidence which has been attributed to clinical conditions associated with the monsoon season.[9] Refrigerated samples are supposed to be kept at room temperature (20-22 °C) prior to analysis. Manufacturer's instructions should be followed regarding the particular preservative and duration of storage.[10]

Aim and objectives

Aim

The aim of the study was to observe significant changes in concentration of sodium, potassium and creatinine urine with different concentrations but with same pH, stored for 60 days, with analyte concentration in reference range and above reference range. The study determined whether patient urine samples can be used for day to day urine quality control.

Objectives

The objectives were the measurement of sodium samples with storage at same pH of around 5 for 60 days in different patient samples (sodium at pH 5.0.); measurement of potassium samples with storage at same pH of around 5 for 60 days in different patient samples. (potassium at pH 5.0) and measurement of creatinine samples with storage at same pH of around 5 for 60 days in different patient samples (creatinine at pH 5.0). The objective was also to observe trends and measure mean and standard deviation in samples sodium, potassium and creatinine in urine with same pH, stored for 60 days, with analyte concentration in normal (unaltered pH and unspiked) urine (reference range).

METHODS

Left over random urine samples of 30 patients were collected from the admitted hospitalized patients from the clinical biochemistry laboratory, New Civil hospital, Surat from 1 December 2020 to 31 January 2021. The study was conducted in New Civil hospital, Surat, which was received for regular urine analysis and aliquoted within a span of 24 hours. The study type was an analytical study and age groups above 18 years regardless of gender, sample was collected. Since a random population was analyzed, so ages above 18 years were taken into account, rather than gender or physical ailments. No ethical clearance was necessary, since all samples were post analytical, left over samples.

Inclusion criteria

The inclusion criteria were the urine samples with volume >10 ml, to ensure that there was sufficient sample for preparation of aliquot and for spiking analytes with normal reference range

Exclusion criteria

The exclusion criteria were the urine samples with analytes volume <10 ml were excluded as the study may not have sufficient sample as planned.

Sample preparation

30 patients urine, approximately 100 ul of left over urine samples without using any preservatives were poured in eppendorf cups, good enough for 60 days, parrafined in eppendorf cups and stored in the deep-fridge of -35 °C.

These aliquots of 30 samples were run every day for 60 days. Results were exported.

RESULTS

Since the samples were randomly picked from the left over samples in biochemistry laboratory, so patient's health status was not taken in account, rather the study was focused on age group only which was kept above 18 years and no gender was kept in account. For each parameter, there were about 60 readings for each analyte. Mean and SD were calculated for each. Data points with difference from mean were considered result were entered in spreadsheet; following was scatter plot of 1st

day of the sample versus average was calculated side by side. The outcome of the study was described with each parameters.

For this parameter, each sample's average and SD were kept at the side which clearly showed the stability of the sample which never even crossed 5 (highest 5, lowest 1.7), even the average SD was 2.6, which again showed the collective stability of the individual 30 samples. As shown in table above, no major deterioration occurred in sodium results after 60 days.

Table 1: 1st day versus average after 60 days (sodium).

S. no.	1st day	Average after 60 days	SD
1.	210.9	215.63	2.8
2.	259.2	253.11	5.0
3.	25.1	25.33	2.2
4.	294.6	295.97	2.4
5.	136.2	136.71	2.1
6.	233.1	237.32	2.7
7.	221.1	225.01	2.2
8.	212.3	216.05	2.3
9.	198.2	196.38	2.4
10.	163.2	164.96	2.7
11.	98.1	96.39	2.4
12.	165.1	165.62	2.0
13.	195.3	195.44	2.2
14.	136.1	137.62	1.7
15.	144.3	146.31	2.6
16.	89.1	89.78	2.6
17.	78.4	77.01	2.6
18.	79.3	77.19	2.6
19.	195.3	196.37	2.4
20.	211.3	215.94	2.3
21.	195.3	196.31	2.5
22.	48.2	45.64	2.6
23.	145.3	146.39	2.5
24.	123.2	122.81	3.1
25.	187.2	185.62	2.7
26.	149.6	145.98	3.4
27.	156.3	156.11	2.2
28.	136.2	136.71	2.1
29.	198.2	196.72	2.3
30.	178.2	175.75	2.9
31.	Average SD		2.6

Linear regression analysis using spread sheet function showed slope of 1.01 and R2 of 0.99. Slope of 1.01 indicated that 1st day of the sample, on comparison to the average of the sample gave only 0.01% higher results.

For this parameter, each sample's average and SD were kept at the side which clearly showed the stability of the sample which never even crossed 5 (highest 3.1, lowest 0.4), even the average SD was 2.4, which again showed the collective stability of the individual 30 samples. As shown in table above, no major deterioration occurred in sodium results after 60 days.

Table 2: 1st day versus average after 60 days (potassium).

S. no.	1st day	Average after 60 days	SD
1.	84.90	85.8	2.6
2.	148.70	146.4	2.5
3.	7.57	7.3	0.4
4.	162.28	165.6	2.1
5.	155.30	156.1	2.2
6.	23.60	24.2	1.5
7.	156.10	155.8	2.1
8.	195.30	196.3	2.5
9.	48.20	45.7	2.6
10.	145.30	146.4	2.5
11.	123.20	122.8	3.1
12.	87.20	86.1	2.5
13.	156.30	156.1	2.2
14.	136.20	136.7	2.1
15.	98.10	96.4	2.4
16.	136.10	137.6	1.7
17.	144.30	146.3	2.6
18.	89.10	89.8	2.6
19.	78.36	77.0	2.6
20.	79.30	77.2	2.6
21.	98.10	96.4	2.4
22.	144.30	146.3	2.6
23.	89.10	89.8	2.6
24.	78.36	77.0	2.6
25.	79.30	77.2	2.6
26.	48.20	45.6	2.6
27.	145.30	146.4	2.5
28.	123.20	122.8	3.1
29.	156.30	156.1	2.2
30.	136.20	136.7	2.1
31.	Average SD		2.4

Linear regression analysis using spreadsheet function showed slope of 1.01 and R2 of 0.99. Slope of 1.01 indicate that 1st day of the sample, on comparison to the average of the sample gave only 0.01% higher results.

For this parameter, each sample's average and SD were kept at the side which clearly showed the stability of the sample which never even crossed 5 (highest 3.4, lowest 1.7), even the average SD was 2.4, which again showed the collective stability of the individual 30 samples. As shown in table above, no major deterioration occurred in sodium results after 60 days.

As shown in Table 3 above, no major deterioration occurred in creatinine results after 60 days.

Table 3: 1st day versus average after 60 days (creatinine).

S. no.	1st day	Average after 60 days	SD
1.	155.3	156.09	2.2
2.	223.4	225.01	2.2
3.	23.6	24.21	1.5
4.	237.4	237.37	2.6
5.	156.1	155.76	2.1
6.	212.3	216.05	2.3
7.	195.3	196.31	2.5
8.	48.2	45.64	2.6
9.	145.3	146.39	2.5
10.	123.2	122.81	3.1
11.	187.2	185.62	2.7
12.	149.6	145.98	3.4
13.	156.3	156.11	2.2
14.	136.2	136.71	2.1
15.	198.2	196.72	2.3
16.	178.2	175.75	2.9
17.	98.1	96.39	2.4
18.	165.1	165.62	2.0
19.	195.3	195.44	2.2
20.	136.1	137.62	1.7
21.	198.2	196.38	2.4
22.	163.2	164.96	2.7
23.	144.3	146.31	2.6
24.	89.1	89.78	2.6
25.	78.4	77.01	2.6
26.	79.3	77.19	2.6
27.	195.3	196.37	2.4
28.	211.3	215.94	2.3
29.	233.1	237.32	2.7
30.	221.1	225.01	2.2
31.	Average SD		2.4

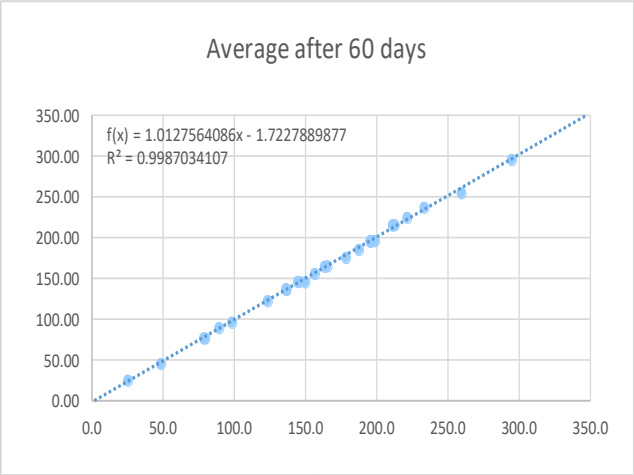

Figure 1: Linear regression analysis.

Linear regression analysis using spreadsheet function shows slope of 1.01 and R2 of 0.99. Slope of 1.01 indicate that 1st day of the sample, on comparison to the average of the sample gave only 0.01% higher results.

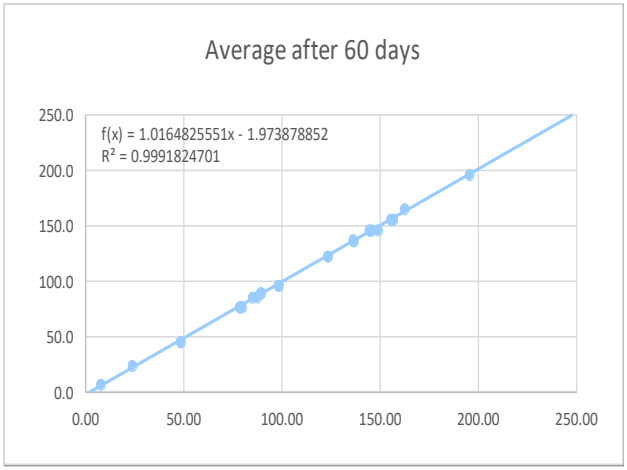

Figure 2: Linear regression analysis.

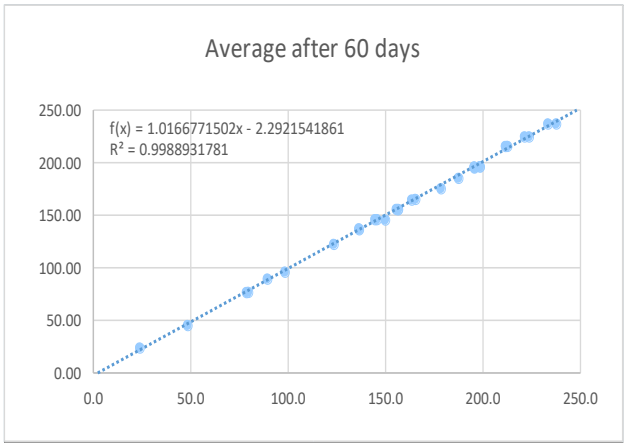

Figure 3: Linear regression analysis.

DISCUSSION

Creatinine

As shown in Table 3, 1st day versus average after 60 days, no major deterioration occurred in sodium results after 60 days. All the sample values which were on the very first day retained even after the 60th day as it was. Also its evident from the Figure 3, linear regression analysis, linear regression analysis using spread sheet function showed slope of 1.01 and R2 of 0.99. Slope of 1.01 indicated that 1st day of the sample, on comparison to the average of the sample gives only 0.01% higher results.

The usefulness of urinary creatinine in measuring the renal function was minimized due to high variations of this measurement, because it's often difficult to obtain an accurate collection of 24 hour urine and its excretion was available, accepted well and a reliable test for muscle mass in healthy people and also for people with CKD.[11,12]

As higher production rates of creatinine and relatively similar excretion rates, men had 21% elevated serum creatinine than women, a fact well known to nephrologists and this caused higher urinary creatinine outputs.[13]

Sodium

As shown in Table 1, 1st day versus average after 60 days, no major deterioration occurred in sodium results after 60 days. All the sample values which were on the very first day retained even after the 60th day as it was. Also its evident from the Figure 1, linear regression analysis, linear regression analysis using spread sheet function showed slope of 1.01 and R2 of 0.99. Slope of 1.01 indicated that 1st day of the sample, on comparison to the average of the sample gave only 0.01% higher results.

Qualitative urinalysis technique using reagent strips was simple and inexpensive; it's performed in most clinical laboratories.[14] Sodium fractional excretion and the renal failure index were accepted as its highly useful and a reliable test which was quite satisfactory sensitivity, specificity and efficiency.[15] SIADH has characteristics of hypotonia, euvolemic hyponatremia with urinary hyperosmolarity occurring from antidiuretic hormone (ADH).[16] Severe hyponatremia was often associated with adverse outcomes as its being recognized that even mild hyponatremia can be associated with patient harm.[17]

Potassium

As shown in Table 2, 1st day versus average after 60 days, no major deterioration occurred in potassium results after 60 days. All the sample values which were on the very first day retained even after the 60th day as it was. Also its evident from the Figure 2, linear regression analysis, linear regression analysis using spread sheet function showed slope of 1.01 and R2 of 0.99. Slope of 1.01 indicated that 1st day of the sample, on comparison to the average of the sample gave only 0.01% higher results.

Constant repetitive measurement of serum potassium was needed to be determined, if hyperkalemia was sustained or even for a transient event.[18] As, GFR decreased and with disturbances of potassium, CKD patients often had co-morbidities that worsened hyperkalemia.[19] Patients with impaired renal function, when GFR was <15 ml/min, increased potassium intake can cause severe hyperkalemia.[20]

Limitation

The study was limited to just a random whole population, the study cannot answer exact queries like ailments related to renal diseases and also can't relate to specific gender groups.

CONCLUSION

As per the final conclusion goes, the patients urine samples can be used as internal quality control sample for at least 60 days for sodium, potassium and creatinine. During the 60 days period, there is no appreciable degradation of samples for sodium, potassium and creatinine measurements. For any level of urine sodium, potassium and creatinine quality control, preservation of urine samples at normal (or neutral) pH 6.8 is effective. Sodium, potassium and creatinine are present in much higher concentration in normal and abnormal urine as compared to concentration available in commercial urine. For these three parameters no pH needs to be adjusted as the urine sample works on neutral pH (5-6) itself and allows monitoring quality of analysis of sodium, potassium and creatinine in clinically relevant ranges. If ever the laboratory has no financial budget to but commercial quality controls form vendors, hereby can use the patients urine sample of desired concentration.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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