In this issue: Improving the preanalytical phase in laboratory medicine

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Impact of chemical preservative in urine samples

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ABSTRACT

Urinalysis is one of the most important tests in the clinical laboratory. In this study we assessed the use of chemical preservative in urinalysis during preanalytical phase. Fifty first morning urine samples from medical laboratory patients were collected and stored with and without chemical preservative.

Difference between medians were analyzed using Wilcoxon signed rank test for glucose, bilirubin, ketones, specific gravity, erythrocytes, pH, proteins, nitrites, leukocytes using urine strips; and on leukocytes, erythrocytes, epithelial cells, and bacteria in the urinary sediment, at 90 minutes after sampling.

Our results showed that the specific gravity and the pH values increased in samples with chemical preservative in urine strip tests.

Concerning urinary sediment analysis no differences were observed in the studied parameters between samples with and without chemical preservative. We suggest that the effect on urine pH is due to the chemical nature of the substances in the preservative.

Thus, we caution about the use of chemical preservatives in samples to be analyzed within short time (i.e. less than 1.5 - 2 hours) after sample collection. Avoid chemical preservatives, in this situation, could help avoid changes in the pH and specific gravity, which could eventually help in maintaining quality in the preanalytical phase of urinalysis.

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INTRODUCTION

Urinalysis is one of the most important tests in the clinical laboratory [1, 2]. Urine contains an enormous amount of information linked to patient's health [3, 4]. The laboratory process consists of three phases: the pre-analytical, analytical and post-analytical phases [5].

The preanalytical phase covers all the procedures before the sample reaches the laboratory to be examined [6, 7]. It encompasses all the steps from sample collection to sample delivery for analysis. Moreover, a pre-preanalytical phase is to be considered mainly related to patient preparation before sample collection [8].

Although most efforts to improve the efficiency in urinalysis have been focused on the analytical phase thus underestimating the preanalytical one, it has been evidenced that up to 60% of errors still occur in the preanalytical phase [9, 10]. In any case the improvements of the analytical phase through automation have led to reduction of up to 10 times the error rate [11,12].

When dealing with urinalysis, both pre-preanalytical and preanalytical phase are to be regarded as critical for the appropriateness of the whole analytical process [3].

As for patient preparation there are several conditions to satisfy that include information on diet, exercise, possible contamination to be avoided by genital cleaning prior to specimen collection, sample collection time (first or second in the morning or casual collection), stream portion of collected sample (first, mid, last), type of sample whether spontaneously collected or by catheterization [1].

One major issue with urine specimens is contamination and consequent microbial growth when sample analysis is delayed due to transportation time. This problem is dealt with by sample refrigeration [13] or by adding chemical preservatives to the urine sample [14].

The use of chemical preservatives has been recommended due to its ability to keep bacterial populations stable for up to 24 hours [9, 10]. In this sense, the aim of this study is to determine the impact of chemical preservative in urine samples.

METHODS

Study design

Fifty urine samples (first morning urine) from the Autonomous University of San Luis Potosi's medical laboratory were included in the analytical study. The BD Vacutainer® Urinalysis Cup Kit was used to collect the sample, which includes a cup to obtain the sample, and a conical tube with sodium propionate 94%, ethyl paraben 5.6% and chlorhexidine 0.4% as preservative.

Patients were asked to transfer urine to the tube with a preservative after collecting the urine sample, and the remaining sample from the cup was also delivered to the laboratory, which was considered as a urine sample without preservative.

This work was performed following the current international ethical guidelines involving human beings for research purposes, adopted by the Declaration of Helsinki [15]. Also, all the participants gave informed consent approved by the Research Ethics Committee from Faculty of Chemistry Science of the Autonomy University of San Luis Potosí (CEID2019-011).

Analysis of urine strips

The standardized chemical analysis for the following parameters: glucose, bilirubin, ketones, specific gravity, erythrocytes, pH, proteins, nitrites, leukocytes was performed on the urine samples using Multistix® 10 SG test strips, and the automated reading system CLINITEK Advantus, both from SIEMENS.

The methods of analysis for each parameter evaluated with urine test strips are shown in Table 1. The analytical performance was verified with SIEMENS CheK-Stix® Combo Pak Control strips for Urinalysis.

Analysis of urinary sediment

The parameters observed in the urinary sediment were leukocytes, erythrocytes, epithelial cells, mucin filaments, crystals and bacteria.

The procedure for urinary sediment analysis was: 8 mL of first morning urine was placed in a conical tube and centrifuged at 400 *g* for 5 minutes [16, 17]. After discarded 7.5 mL of the supernaµL of this re-suspended pellet was placed on a slide, covered with a coverslip (18 x 18 mm2) then observed under the microscope using 100X and 400X objective [18].

Table 1 Methods of analyses

Parameter	Method
Glucose	Glucose oxidase
Bilirubin	Union of bilirubin with dichloroaniline diazotized in an acidic medium
Ketones	Colorimetric reaction between acetoacetic acid and nitroprusside
Specific gravity	Change of pKa in polyelectroliters in relation to ionic concentration
Blood	Hemoglobin pseudoperoxidase activity that catalyzes the reaction of diisopropylbenzene dihydroperoxide with 3, 3', 5, 5'-tetramethylbenzidine
pH	Combination of methyl red and blue bromothymol that react with hydrogen ions
Protein	Protein error of indicators
Urobilinogen	Reaction of ρ-diethylaminobenzaldehyde with urobilinogen in acidic medium
Nitrites	Griess assay principle. The reaction reveals the presence of nitrite and therefore, indirectly, the existence of bacteria forming it in the urine
Leukocytes	Esterases that catalyze the hydrolysis of the pyrrolic amino acid ester

Statistical analysis

For the statistical analysis the Wilcoxon signed rank test was used to evaluate the difference between urine samples with and without chemical preservatives; the established statistical significance was set at $p \leq 0.05$. The statistical package used was the software SPSS Statistics® 20.

RESULTS

We found that the urine samples with chemical preservative showed higher values of specific gravity and pH in comparison with the samples without chemical preservative. As for the remaining parameters no differences were observed between the medians of samples with and without chemical preservative. And in addition to this, we did not observe significant differences between the figurate elements of the urinary sediment in the presence or absence of chemical preservative (Table 2).

DISCUSSION

In this work we evaluated the effect of chemical preservative in the preanalytical phase and we found that the specific gravity and the pH were both lowered in the absence of chemical preservative at 90 minutes after sample collection. Chemical preservatives are available to maintain sample integrity without cell lysis, to avoid bacterial growth, or *in vitro* crystal formation [9,14,19]. Moreover, preservatives can affect some parameters such as leukocyte esterase, glucose and proteins [20, 21, 22].

According to the increase of the pH and specific gravity values in the samples with chemical preservative (Table 2), the question arises if such changes regarding pH and specific gravity are clinically relevant, thus being able to influence the interpretation of urinalysis. For example, one possible implication of increased specific gravity due to preservative could jeopardize the diagnosis of pseudohyposthenuria in the pediatric population, whose values of specific gravity are lower than normal children [23, 24]. In addition, the pH may affect the concentration of certain urinary parameters [21].

Although samples without chemical preservative showed a reduced specific gravity in respect of added preservative, when evaluating the figurated elements such as cells and microorganisms, we did not find any differences between the samples with or without chemical preservative (Table 2).

Therefore, we can say that although the chemical preservative increased specific gravity, this did not affect the microscopic analysis of urine.

It has been reported that most of the parameters evaluated during urinalysis depend strongly on the time window between sampling and analysis. Moreover, morphological studies showed a higher reproducibility when time was between 1 and 2 hours [25]. Thus, the use of chemical preservative is recommended for those samples that will be processed after two hours of collection [21,22].

The optimal time for performing the urinalysis and the impact of the chemical preservative on urine samples has been previously studied by Dolscheid-Pommerich *et al*. [26]. They found a significant decrease in concentrations of erythrocytes and leukocytes between 90 and 120 minutes after sample collection, in samples stored at room temperature [26]. However, they did not find changes in pH and specific gravity before 120 minutes of collection; the authors recommend 90 minutes, as an optimal time for the urinalysis to be performed after the collection of the sample [26].

On the other hand, the changes that chemical preservatives can cause on the different parameters of urinalysis have also been studied; Delanghe *et al*., described that chlorhexidine can causes an alteration in glucose and pH parameters [21].

We suggest that the effect on urine pH (Table 2) is due to the chemical nature of the substances in the preservative and that this change in pH is related to the increase in specific gravity, thus explaining possible changes in these parameters even in short time windows.

In conclusion, given the unclear consensus on the optimal time for the use of chemical preservatives, and based on our findings, we caution about the use of chemical preservatives in samples to be analyzed within short time (i.e. less than 1.5 - 2 hours) after sample collection.

Avoid chemical preservatives, in this situation, could help avoid changes in the pH and specific gravity, which could eventually help in maintaining quality in the preanalytical phase of urinalysis.

Table 2 Impact of chemical preservative in urine samples

Parameter	Frequency (N = 50)		*p*
	Samples with chemical preservative	Samples without chemical preservative	
Urine strip			
Glucose			
Absent	49/50	49/50	> 0.05
100	1/50	1/50	
Bilirubin			
Absent	50/50	50/50	> 0.05
Ketones			
Absent	50/50	50/50	> 0.05
Specific gravity			
1.005	1/50	4/50	**0.001**
1.010	7/50	4/50	
1.015	3/50	7/50	
1.020	11/50	13/50	
1.025	13/50	9/50	

1.030	15/50	13/50	
Blood			
Absent	39/50	37/50	> 0.05
Erythrocytes			
10	6/50	9/50	
Free hemoglobin			
10	1/50	0/50	
25	2/50	2/50	
80	1/50	0/50	
200	1/50	2/50	
pH			
5.0	1/50	17/50	**0.001**
6.0	39/50	26/50	
6.5	9/50	5/50	
7.0	1/50	2/50	
Proteins			
Absent	49/50	50/50	> 0.05
< 30	1/50	-	
Urobilinogen			
0.2	50/50	50/50	> 0.05
Nitrites			
Absent	45/50	45/50	> 0.05
Present	5/50	5/50	
Leukocytes			
Absent	42/50	44/50	

15	2/50	1/50	> 0.05
70	5/50	4/50	
125	1/50	1/50	
Microscopic analysis			
Leukocytes/high power field			
0-5	44/50	44/50	> 0.05
6-10	5/50	5/50	
11-25	1/50	1/50	
Erythrocytes/high power field			
0-2	49/50	49/50	> 0.05
6-10	1/50	1/50	
Epithelial cells			
Absent	20/50	20/50	> 0.05
Low	24/50	25/50	
Moderate	5/50	4/50	
Abundant	1/50	1/50	
Mucin filament			
Absent	15/50	15/50	> 0.05
Low	24/50	24/50	
Moderate	9/50	9/50	
Abundant	2/50	2/50	
Bacteria			
Low	38/50	38/50	> 0.05
Moderate	6/50	6/50	

Abundant	6/50	6/50	
Crystals			
Absent	37/50	36/50	> 0.05
Low	9/50	10/50	
Moderate	3/50	3/50	
Abundant	1/50	1/50	

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