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The role of tubes with preservative in urinalysis of pregnant women

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Abstract

In order to increase the efficiency of the preanalytical process at the Public Health Laboratory (PHL) where urinalysis is performed on pregnant women admitted at primary care family health centres (FHC), our aim was to demonstrate the efficacy of preservative urine tubes in this study. The first morning urine samples of 84 pregnant women were collected into urine tubes with or without preservative. The first analysis was used as a reference. The tubes with preservative containing chlorhexidine were transported at room temperature and analysed on the 2, 6, 24, and 72 h. Tubes with or without preservative and transported according to cold-chain principles were analysed on the 2nd and 6th h. Changes in comparison to the reference tube were analysed. When the test results of the tubes with preservative and kept at room temperature were compared to those of the reference tubes, the only changes that were observed were in the leukocyte morphology. During microscopic examination of the tubes with or without preservative and transported in accordance with cold-chain principles, no differences were found except pH changes during strip tests and changes in the crystal structure. The tubes with preservative containing chlorhexidine, whose performance was tested with the aim of controlling pre-analytical variables, may be used in conditions in which transportation in accordance with cold-chain principles is impossible. Tubes without preservative were observed to be adequate in conditions in which transportation in accordance with cold-chain principles is possible within 6 h.

Keywords: Tubes with preservative, pregnancy, pre-analytical phase

Introduction

Urinalysis is the third most frequently performed diagnostic test in clinical laboratories [1,2]. Prolongation of the period between the urine sample collection and the analysis (> 2 h), the inability to control the temperature, and an absence of preservative decrease the quality of the samples [3,4].As urine samples analysed at the Public Health Laboratory are transported by vehicle from family health centres (FHC) located far away, the period between obtaining the sample and the analysis may exceed 2 h. While transporting the samples in accordance with cold-chain principles may prevent bacterial growth and cell destruction or structural deterioration, but also negative effects may be observed on the crystal structure and other particles [1,5,6].

Use of stabilizers such as ethanol and polyethylene glycol may prevent metabolic changes and bacterial growth [3, 5]. Urinalysis is important during primary care follow-up of pregnant women and is considered to be one of the most important criteria in the evaluation of healthcare systems' activities. It is imperative to minimize the effects of preanalytical factors for a precise and reliable analytical phase. The aim of this study was to assess the stability of urine samples for up to 72 h at room temperature in tubes containing preservative material and to compare tubes that either with or without preservatives in a cold environment in order to demonstrate their performance.

Material and methods

Subjects

The first morning urine samples of 84 pregnant women admitted at ten FHCs in Uşak City between June and September 2015 were obtained.

Methods

Approximately 90 mL urine samples were collected from each pregnant woman and were portioned into 9 tubes

1. Three polystyrene tubes that did not contain preservatives for 0 h were used as the reference tube; these were analyzed at the 2 and 6 h and transferred according to cold chain principles, with approximately 8 mL in each tube).

2. Urine tubes containing preservative (polyethylene terephthalate

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[PET] plastic with chlorhexidine-based preservative) were tested at the 2nd h and 6th h (transferred according to cold-chain principles, with approximately 8 mL in each tube).

3. Urine tubes containing preservative (polyethylene terephthalate [PET] plastic with chlorhexidine-based preservative) were tested at the 2, 4, 24, 72 h (with approximately 8 mL in each tube).

The tubes that we evaluated in this study were "The Becton-Dickinson Vacutainer® Plus Urinalysis Preservative (BD UAP) Tube" (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), containing 0.4% chlorhexidine, 5.6% ethyl paraben, and 94% sodium propionate. All 0 h urine samples were evaluated at the FHC by the same person at the time of obtaining the urine samples. This evaluation was considered to be the reference, and comparisons with other tubes were done according to this reference.

The other urine tubes were transported to the Public Health Laboratory by means of transport vehicles. A total of 4 samples with macroscopic hematuria were excluded, and 84 samples were analysed. Manual microscopic analysis of the urine samples was performed after macroscopic (colour and appearance), physical, and chemical analysis [7]. Chemical analysis was performed with LabStrip Urinalysis U11 Plus strips manually. Decant procedure was performed in a manual microscopic examination with 0.5 mL residue at the tube bottom. The sediment was evaluated under a microscope using 22x22 mm slides (with x40 objectives). A total of 5 areas were evaluated under the microscope, according to international standards, and their means were calculated.

The results were presented as /HPF (high power field). Strip and microscopic examination results were compared, and strip examination was repeated when the two results were not concordant. The cut-off for a positive result was considered as leukocyte >5, epithelium >5, bacteria >10, and erythrocytes >3.

Written informed consents were obtained from all participants. The study was approved by the Ethics Committee of Pamukkale University, Non-Interventional Clinical Investigations.

Statistical analysis

The urine samples analysed at 0 h were considered to be the reference, and the percentage accordance changes in the other tubes were calculated in comparison with the reference tube. Accordance over 80% was considered to be "good." The 95% confidence intervals used for the accordance were calculated according to CLSI document EP12-A2.

Results

The mean age of the 84 pregnant women included in this study was 27.96 years (range, 17–44 years). A total of 15 of them were in the 1st trimester, 33 were in the 2nd trimester, and 36 were in the 3rd trimester. Analysis of 756 urine samples of the 84 pregnant women was manually performed. According to manual strip test results, leukocytes were positive in 41.7%, red blood cells were positive in 5%, nitrite was positive in 9.5%, glucose was positive in 2.4%, and ascorbic acid was positive in 10.7%. Positive results were not obtained for bilirubin, urobilinogen, ketone, or protein. The mean density was 1020 and the mean pH was 6.98.

According to the manual microscopic examination, leukocytes were found in 52.4%, red blood cells were found in 6%, bacteria were found in 45.2%, amorphous urate was found in 39.3%, squamous epithelium was found in 69%, maya cells were found in 1.2%, and calcium oxalate crystals was found in 4.8% (Ca-Ox). In the comparison of tubes with preservative kept at room temperature and transported to the PHL with the reference tubes, no differences were found except for the leukocyte counts and the morphology at the 72nd h. According to microscopic examination, the disruption of cell walls and changes in normal morphology were also observed.

However, leukocytes were still present. In Table 1, the only parameter of the tubes with preservative at room temperature that differed in comparison with the reference tube was the percentage of the leukocyte counts. Except for this, no differences were found in the chemical strip and microscopic analysis. In polystyrene tubes transported in accordance with cold-chain principles that did not contain preservatives and tubes that did contain preservative, amorphous urates were found to change into a light pink precipitate, suggesting amorphous phosphate due to cold, and pH was found to have increased in the same tubes. During a microscopic examination, there was also observed to be amorphous phosphate. Hence, the negative effects of a cold environment on the crystal structure and pH were detected in the polystyrene tubes that did not contain preservative and the tubes that did contain preservative.

In 3 of the tubes transported and kept in accordance with cold-chain principles, leukocyte changes were found in strip tests, and structural disruptions in leukocyte morphology were also observed in some areas under microscopic examination (Table 2, 3). The results for the tubes with or without preservative and transported according to cold-chain principles were similar. No changes except those shown in the tables were detected. Accordance with the reference tube was 100% (95% CI 92.5-100).

Table 1. Concordance evaluation of WBCs parameter in tubes with preservative kept at room temperature

Microscopy	Microscopy 2h % agreement (95% CI)	Microscopy 6h % agreement (95% CI)	Microscopy 24h % agreement (95% CI)	Microscopy 72h % agreement (95% CI)
WBC s	WBC s %100 (92.5-100)	WBC s %100 (92.5-100)	WBC s %100 (92.5-100)	WBC s %71,43 (56.4-67.5)

Table 2. Concordance evaluation of chemistry parameters in refrigerated polystyrene tubes and tubes with preservative with freezer packs

Chemistry	2h % agreement (95% CI)	6h % agreement (95% CI)	2h % agreement (95% CI)	6h % agreement (95% CI)
pH	%100 (92.5-100)	%67.9 (57.3-76.8)	%100 (92.5-100)	%67.9 (57.3-76.8)
Leukocytes (leu/ml)	%100 (92.5-100)	%96.5 (90-98.8)	%100 (92.5-100)	%96.5 (90-98.8)

Table 3. Concordance evaluation of microscopic analysis parameters in refrigerated polystyrene tubes and tubes with preservative with freezer packs

Microscopy	2h% agreement (95% CI)	6h% agreement (95% CI)	2h% agreement (95% CI)	6h% agreement (95% CI)
WBCs	%96.5 (90-98.8)	%96.5 (90-98.8)	%96.5 (90-98.8)	%96.5 (90-98.8)
Amorphous urate	%100 (92.5-100)	%60.7 (67.41-85.1)	%100 (92.5-100)	%60.7 (67.41-85.1)

Discussion

Complications of urinary tract infections during pregnancy are observed in a broad clinical spectrum, from asymptomatic bacteriuria to serious pyelonephritis [8]. In the study by Laura A. Schieve et al, the risk of premature birth and preterm low-weight birth was presented to have increased in women who experience urinary tract infections during pregnancy [9]. For this reason, routine urinalysis and urine culture examinations done by family physicians in each trimester are recommended [10,11].

Urine samples of pregnant women are transported to public health laboratories from

FHCs, but these cannot be examined in 2 h, and the quality of the samples may decrease [3]. Studies have reported the hypothesis that preservative urine tubes may prevent loss of sample quality during waiting [12]. We compared tubes containing or not containing preservatives and investigated the performance of these tubes in this study on pregnant women.

The urine samples were examined manually by microscope in our study, which is the gold standard [13]. Bacterial growth may be prevented in urine tubes with or without preservative when they are transported and kept according to cold-chain principles. However, cold preservation has caused changes in the crystal structure and leukocyte morphology.

Similarly, in a study by Ercan et al, changes in leukocyte morphology were detected, and negative effects of cold preservation on the crystal structure were observed [5]. Mostly, amorphous urate was found in the urine samples of our volunteers.

The colour of the amorphous urate changed to pink at the end of the 6th h in a cold environment (like amorphous phosphate). This was due to pH changes in the urine samples. However, change from amorphous urate to amorphous phosphate was not observed in urine samples kept in tubes with preservative at room temperature. We found changes in very few parameters in tubes with and without preservative, both transported and kept according to cold chain or at room temperature, possibly due to the analysis being performed by the same person. In the study by Kouri et al with BD UAP tubes with preservative, results with more accordance were obtained in strip tests of samples kept at room temperature compared to samples kept according to cold-chain [14]. In our study, no changes were observed in the strip tests of the samples that were kept in tubes with preservative kept at room temperature, while changes were found in the pH and leukocyte results in the samples kept in a cold environment. Another point that we detected in our study is that it is necessary to mix the urine samples with preservative materials by turning the tube upside down gently a few times.

Conclusion

In conclusion, the quality of the urine samples may be preserved for analysis for up to 6 h in tubes that do not contain preservative material when transportation of the samples is possible in cold-chain conditions. It was observed that tubes containing preservative were appropriate for locations such as the Public Health Laboratory where samples can sometimes take over 2 h to be transported and where cold-chain transportation is not available.

Competing interests
The authors declare that they have no competing interest.
Financial Disclosure
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References

1. Coppens A, Speeckaert M, Delanghe J. The pre-analytical challenges of routine urinalysis. Acta Clin Belg. 2010;65:182-9.
2. Delanghe J, Speeckaert M, Preanalytical requirements of urinalysis. Biochem Med (Zagreb). 2014;24:89-104.
3. Lippi G, Becan-McBride K, Behulova D, et al. Preanalytical quality improvement: in quality we trust. Clin Chem Lab Med. 2013;51:229-41.
4. Clinical and laboratory standards institute. Urinalysis and collection, transportation,and preservation of urine specimens. Approved Guideline-Third Edition NCCLS document GP16-A3. 2013 November 11t
5. Ercan M, Akbulut ED, Abuşoğlu S, et al. Stability of urine specimens stored with and without preservatives at room temperature and on ice prior to urinalysis. Clin Biochem. 2015;48:919-22.
6. Manoni SV, Caleffi A, Alessio MG, et al. Stability of common analytes and urine particles stored at room temperature before automated analysis RIMeL / IJLaM 2008;192-8.
7. Bottini VP. Mayara H, Martinez M, et al. Urinalysis: Comparisonbetween Microscopic Analysis and a New Automated Microscopy Image- Based Urine Sediment Instrument Clin. Lab. 2014;60:693-7.
8. Cunningham FG, Lucas MJ, Urinary tract infections complicating pregnancy. Baillieres Clin Obstet Gynaecol. 1994;8:353-73.
9. Schieve A. Laura, Handler A, Hershow R, et al. Urinary Tract Infection during Pregnancy: Its Association with Maternal Morbidity and Perinatal Outcome. Am J Public Health. 1994;84:405-10.
10. Murray N, Homer CSE, Davis GK, et al. Theclinical utility of routine urinalysis in pregnancy:a prospective study. Med J Aust. 2002;177:477-80.
11. Kirkham C, Harris S, Grzybowski S. Evidence-based prenatal care: part I.General prenatal care and counseling issues. Am Fam Physician. 2005;71:1307-16.
12. Eisinger SW, Schwartz M, Dam L, et al. Evaluation of the BD vacutainer plus urine C&S preservative tubes compared with nonpreservative urine samples stored at 4°C and room temperature. Am J Clin Pathol. 2013;140:306-13.
13. Langlois RM, Delanghe JR, Steyaert SR, et al. Automated flow cytometry compared with an automated dipstick readerfor urinalysis. Clin Chem. 1999;45:118-22.
14. Kouri T, Malminiemi O, Penders J, et al. Limits of preservation of samples for urine strip tests and particle counting. Clin Chem Lab Med. 2008;46:703-13.