Clinical Biochemistry 48 (2015) 919–922

Contents lists available at ScienceDirect

Clinical Biochemistry

journal homepage: www.elsevier.com/locate/clinbiochem

Short Communication

# Stability of urine specimens stored with and without preservatives at room temperature and on ice prior to urinalysis

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ARTICLE INFO

*Article history:*
Received 10 February 2015
Received in revised form 14 May 2015
Accepted 19 May 2015
Available online 27 May 2015

*Keywords:*
Preanalytic phase
Urinalysis
Preservative tube

ABSTRACT

**Objectives:** Laboratories determine the most appropriate approach for the collection and transport of urine specimens. We investigated the effect of a chlorhexidine-based preservative tube on sample stability, compared the results of refrigerated polystyrene tubes with no additives, and investigated the effect of temperature on the performance of preservative tubes.

**Design and methods:** Fresh urine specimen (n = 48) aliquots in BD Vacutainer® Plus Urinalysis Preservative Tubes and polystyrene tubes were analyzed on an Iris Diagnostics iQ200. Samples in polystyrene tubes were refrigerated for 4 and 8 h. Four aliquots in preservative tubes were kept at room temperature for 4, 8, 24, and 72 h, while two aliquots were kept on ice for 4 and 8 h.

**Results:** There was good agreement for all chemistry and microscopy parameters with the exceptions of white blood cells (WBCs) at 24 and 72 h and red blood cells (RBCs) at 72 h. Preservative tubes on ice showed a significant decrease in concordance of WBCs and calcium oxalate (CaOx) parameters compared with the results at room temperature. Results of refrigerated polystyrene tubes showed good agreement with the exceptions of WBC clumps and amorphous crystal at 8 h.

**Conclusions:** A chlorhexidine-containing preservative tube seems advantageous for urine sample transport from outside healthcare services. A preservative tube offers comparable results with urine samples kept in a refrigerator for 4–8 h for the majority of parameters. Keeping samples at room temperature is recommended when preservative tubes are used because ice produces a negative effect on WBCs and CaOx.



## Introduction

Urinalysis is one of the most common screening tests performed in the clinical laboratory [1,2]. According to CLSI GP16A3 Guidelines [3], the recommended analysis time for urine samples is within 2 h of collection. For delayed analysis, keeping the sample in an uncontrolled temperature environment or the lack of a preservative will reduce the quality of the results [4]. Refrigeration is known to reduce cell degradation and bacterial growth, but the increased frequency of crystals may affect the recognition of other particles [5]. Commercial preservative tubes are available to maintain sample integrity without cell lysis, bacterial growth, or in vitro crystal formation [3].

The Becton–Dickinson Vacutainer® Plus Urinalysis Preservative (BD UAP) Tube (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) contains 0.4% chlorhexidine, 5.6% ethyl paraben, and 94% sodium propionate. The manufacturer states that the tube stabilizes urine over 72 h without the need for refrigeration and does not interfere with strip and microscopy analysis on various urine analyzers.

The primary aim of this study was to assess the effect of the BD UAP tube on the stability of urine aliquots kept at room temperature for up to 72 h. Our secondary and tertiary aims were to investigate whether temperature affected the performance of the preservative tube and to compare these options with polystyrene test tubes refrigerated for up

*Abbreviations:* CLSI, Clinical and Laboratory Standards Institute; BD, Becton Dickinson; PET, Polyethylene terephthalate; RBCs, Red blood cells; WBCs, White blood cells; CaOx, Calcium oxalate; SG, Specific gravity; HPF, High power field.

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http://dx.doi.org/10.1016/j.clinbiochem.2015.05.016
0009-9120/

to 8 h in order to obtain data on the appropriate preservation of urine specimens.

## Materials and methods

### *Urine specimens*

Forty-eight first-voided morning urine samples from the Outpatient Clinics of Urology submitted to the Central Laboratory of the Ankara Numune Education and Research Hospital were included in the study. Approximately 90 mL was obtained per patient, and specimens with macroscopic hematuria were excluded. All the participants gave informed consent approved by the Ethics Committee of the Ankara Numune Education and Research Hospital.

### *Study procedure and time points*

Urine specimens were divided into the following tubes for particle and test strip analyses:

1. Preservative tube (polyethylene terephthalate [PET] plastic with chlorhexidine-based preservative) at room temperature, four aliquots (8 mL) for analyses at 4, 8, 24, and 72 h;
2. Preservative tube (PET plastic with chlorhexidine-based preservative) in a container with ice packs, two aliquots (8 mL) for analyses at 4 and 8 h;
3. Polystyrene test tube with no additives, refrigerated, two aliquots (8 mL) for analyses at 4 and 8 h.

### *Measurements*

Chemical analysis and microscopy analysis were performed with iChem® VELOCITY™ Strips on an iChem® VELOCITY™ Urine Chemistry System, iQ®200 SPRINT™ (Iris Diagnostics Inc., Chatsworth, CA, USA) by reflectance photometry and flow cell digital imaging; the Auto-Particle Recognition software classifies each image into one of 12 categories [6]. Quality control materials (iChemVELOCITY IRISpec CA/CB/CC [Iris Diagnostics]) were run daily. A bimonthly external quality assurance program (RIQAS [Randox International Quality Assessment Scheme] Urinalysis Program, [Randox Laboratories-US, Ltd.]) was also used by the laboratory.

Limits of positivity were accepted as: 3, 5, and 4 cells per high-power field (HPF) for RBCs, WBCs, and squamous epithelial cells, respectively. Any detected yeast, cast, uric acid crystal, and WBC clump per HPF was accepted as positive.

### *Statistical analysis*

Statistical analysis was performed by using SPSS v.21 software. Concordance between each aliquot and the 0 h aliquot was estimated as percentage (95% CI) +/− one color block from the best fit line or within one grading difference. Higher than 80% concordance was accepted as good agreement. The 95% confidence intervals for agreement were calculated according to CLSI document EP12-A2.

## Results

For urine chemistry parameters, 4.1%, 35.4%, 68.8%, 2.1%, and 56.3% of the 0-h samples were positive for glucose, protein, blood, nitrite, and leukocyte esterase, respectively. Bilirubin, urobilinogen, ketone, and pH results did not exceed reference intervals. Percentages exceeding the cut-off value for RBCs, WBCs, WBC clump, epithelial cells, casts, yeast, and CaOx crystals were 52.8%, 50%, 6.3%, 4.2%, 2.1%, 6.3%, and 8.3%, respectively. For bacteria, amorphous and uric acid crystal parameters, results were within the reference interval. The mean relative density and pH of urine specimens in the polystyrene test tubes were 1.017 and 5.0, respectively, while the corresponding values were 1.017 and 5.5 for the samples in preservative tubes.

**Table 1**
Concordance evaluation of chemistry and microscopy parameters in preservative tubes kept at room temperature.

| Chemistry | 4 h % agreement (95% CI) | 8 h % agreement (95% CI) | 24 h % agreement (95% CI) | 72 h % agreement (95% CI) |
|---|---|---|---|---|
| Glucose | 97.9% (89.1–99.6) | 97.9% (89.1–99.6) | 97.9% (89.1–99.6) | 95.8% (92.5–100.0) |
| Protein | 97.9% (89.1–99.6) | 93.8% (83.2–97.8) | 95.8% (86.0–98.8) | 97.9% (89.1–99.6) |
| Blood | 100.0% (92.5–100.0) | 97.9% (92.5–100.0) | 100.0% (92.5–100.0) | 93.8% (83.2–97.8) |
| Ketone | 100.0% (92.5–100.0) | 97.9% (86.0–98.8) | 100.0% (92.5–100.0) | 97.9% (86.1–98.8) |
| Nitrite | 100.0% (92.5–100.0) | 100.0% (92.5–100.0) | 100.0% (92.5–100.0) | 97.9% (92.5–100.0) |
| Leukocyte esterase | 93.8% (89.1–99.6) | 93.8% (89.1–99.6) | 89.6% (77.8–95.5) | 87.5% (75.3–94.1) |
| pH | 97.9% (89.1–99.6) | 97.9% (89.1–99.6) | 100% (92.5–100.0) | 97.9% (86.1–98.8) |
| SG | 93.8% (89.1–99.6) | 93.8% (83.2–97.8) | 93.8% (89.1–96.4) | 93.8% (89.1–96.4) |
| **Microscopy** | **4 h % agreement (95% CI)** | **8 h % agreement (95% CI)** | **24 h % agreement (95% CI)** | **72 h % agreement (95% CI)** |
| RBCs | 89.5% (71.0–90.7) | 91.6% (80.6–96.7) | 87.5% (86.0–98.8) | 79.2% (75.3–94.1) |
| WBCs | 95.8 % (89.1–99.6) | 97.9% (86.0–98.8) | 70.8% (59–83.4) | 77.1% (63.5–86.8) |
| WBC clump | 95.8% (86.0–98.8) | 97.9% (89.1–99.6) | 97.9% (89.1–99.6) | 97.9 % (89.1–99.6) |
| Epithelial cells | 97.9% (92.5–100.0) | 93.8% (89.1–99.6) | 85.4% (83.1–97.9) | 89.6% (89.1–99.6) |
| Casts | 97.9% (89.1–99.6) | 93.8% (89.1–99.6) | 95.8% (92.5–100.0) | 95.8% (92.5–100.0) |
| Bacteria | 100.0% (92.5–100.0) | 97.9% (92.5–100.0) | 93.8% (86.0–98.8) | 95.8% (86.0–98.8) |
| Yeast | 100.0% (92.5–100.0) | 100.0% (92.5–100.0) | 100.0% (92.5–100.0) | 97.9% (86.0–98.8) |
| Amorphous crystal | 97.9% (92.5–100.0) | 97.9% (92.5–100.0) | 100.0% (92.5–100.0) | 100.0% (92.5–100.0) |
| CaOx crystal | 91.6% (83.3–97.7) | 91.6% (83.3–97.7) | 91.6% (83.2–97.8) | 81.3% (68.1–89.8) |
| Uric acid | 91.6 % (80.4–96.7) | 91.6 % (80.4–96.7) | 91.6 % (80.4–96.7) | 91.6 % (80.4–96.7) |

In the preservative tube, there was good agreement for all the chemistry and microscopy parameters at the different time intervals with the exceptions of WBCs at 24 and 72 h and RBCs at 72 h. Concordance at 4 and 8 h was good for WBCs but concordance was moderate at 24 and 72 h (70.8%, 77.1%, respectively). For RBCs, agreement was good at 4, 8, and 24 h, whereas it was moderate at 72 h (79.2%) (Table 1).

The effect of temperature on samples was then examined in order to determine appropriate transport conditions between the outside healthcare services and the Public Health Laboratory. As indicated in Table 2, keeping preservative tubes on ice caused a significant decrease in the concordance of WBCs and CaOx parameters. For the refrigerated polystyrene tube samples with no additives, particle and chemistry analysis showed good agreement with the reference group with the exceptions of WBC clump and amorphous crystals at 8 h (77.1% and 79.1% concordance, respectively).

## Discussion

The results of the study obtained data for the accurate analysis of the urine specimens with different storage conditions.

Concordance evaluation indicated good agreement for all the chemistry and microscopy parameters with the exceptions of WBCs at 24 and 72 h and RBCs at 72 h. Possible changes in cell morphology, which

**Table 2**
Concordance of preservative tubes kept at room temperature (PTRT), preservative tubes on freezer packs (PTWFP), and refrigerated polystyrene tubes (with no additives) (RPT) compared with the 0-h group.

| | PTRT | | PTWFP | | RPT | |
|---|---|---|---|---|---|---|
| Microscopy | 4 h % agreement (95% CI) | 8 h % agreement (95% CI) | 4 h % agreement (95% CI) | 8 h % agreement (95% CI) | 4 h % agreement (95% CI) | 8 h % agreement (95% CI) |
| RBCs | 89.5% (71.0–90.7) | 91.6% (80.6–96.7) | 95.8% (83.1–97.8) | 81.3% (76.9–96.3) | 85.4% (77.8–95.5) | 85.4% (71.9–97.5) |
| WBCs | 95.8 % (89.1–99.6) | 97.9% (86.0–98.8) | 60.4% (50.4–76.6) | 52.1% (41.5–69.3) | 89.5% (70.4–91.3) | 93.7% (83.2–97.8) |
| WBC clump | 95.8% (86.0–98.8) | 97.9% (89.1–99.6) | 91.6% (77.8–95.5) | 91.6% (77.8–95.5) | 81.3% (66.4–87.6) | 77.1% (66.4–87.6) |
| Epithelial cells | 97.9% (92.5–100.0) | 93.8% (89.1–99.6) | 97.9% (89.1–99.6) | 95.8% (89.1–99.6) | 97.9% (86.0–98.8) | 95.8% (92.5–100.0) |
| Casts | 97.9% (89.1–99.6) | 93.8% (89.1–99.6) | 97.9% (89.1–99.6) | 97.9% (89.1–99.6) | 97.9% (84.1–96.8) | 97.9% (84.1–96.8) |
| Bacteria | 100.0% (92.5–100.0) | 97.9% (92.5–100.0) | 95.8% (83.2–97.8) | 97.9% (89.1–99.6) | 95.8% (87.2–97.7) | 100.0% (92.5–100.0) |
| Yeast | 100.0% (92.5–100.0) | 100.0% (92.5–100.0) | 95.8% (86.0–98.8) | 97.9% (89.1–99.6) | 97.9% (89.1–99.6) | 95.8% (86.0–98.8) |
| Amorphous crystal | 97.9% (92.5–100.0) | 97.9% (92.5–100.0) | 95.8% (86.0–98.8) | 97.9% (89.1–99.6) | 89.5% (77.8–95.5) | 79.1% (65.7–88.3) |
| CaOx crystal | 91.6% (83.3–97.7) | 91.6% (83.3–97.7) | 87.5% (83.2–97.8) | 87.5% (83.2–97.8) | 95.8% (86.0–98.8) | 91.6% (80.4–96.7) |
| Uric acid crystal | 91.6 % (80.4–96.7) | 91.6 % (80.4–96.7) | 97.9% (89.1–99.6) | 97.9% (89.1–99.6) | 100.0% (92.5–100.0) | 100.0% (92.5–100.0) |
| Chemistry | 4 h % agreement (95% CI) | 8 h % agreement (95% CI) | 4 h % agreement (95% CI) | 8 h % agreement (95% CI) | 4 h % agreement (95% CI) | 8 h % agreement (95% CI) |
| Glucose | 97.9% (89.1–99.6) | 97.9% (89.1–99.6) | 95.8% (89.1–99.6) | 95.8% (89.1–99.6) | 97.9% (89.1–99.6) | 97.9% (89.1–99.6) |
| Protein | 97.9% (89.1–99.6) | 93.8% (83.2–97.8) | 91.6% (83.2–97.8) | 91.6% (83.2–97.8) | 100.0% (92.5–100.0) | 97.9% (86.0–98.4) |
| Blood | 100.0% (92.5–100.0) | 100.0% (92.5–100.0) | 95.8% (92.5–100.0) | 97.9% (89.1–99.6) | 97.9% (89.1–99.6) | 91.6% (80.4–96.7) |
| Ketone | 100.0% (92.5–100.0) | 100.0% (92.5–100.0) | 97.9 % (89.1–99.6) | 97.9 % (89.1–99.6) | 100.0% (92.5–100.0) | 100.0% (92.5–100.0) |
| Nitrite | 100.0% (92.5–100.0) | 97.9% (92.5–100.0) | 100.0% (92.5–100.0) | 100.0% (92.5–100.0) | 95.8% (86.1–98.8) | 93.7% (83.0–97.8) |
| Leukocyte esterase | 100.0% (92.5–100.0) | 97.9% (86.0–98.8) | 95.8% (86.0–98.8) | 91.6% (83.2–97.8) | 95.8% (89.1–99.6) | 100.0% (92.5–100.0) |
| pH | 100.0% (92.5–100.0) | 100.0% (92.5–100.0) | 97.9 % (92.5–100.0) | 97.9% (92.5–100.0) | 97.9% (89.1–99.6) | 97.9% (89.1–99.6) |
| SG | 93.8% (89.1–99.6) | 93.8% (89.1–99.6) | 93.8% (89.1–99.6) | 93.8% (89.1–99.6) | 93.8% (89.1–99.6) | 93.8% (89.1–99.6) |

affected the accurate detection and identification of these cells, is one possible explanation for this. No increase was found in crystal quantity, which is an important error source leading to misclassification as erythrocytes on automated urine analyzers.

Miller demonstrated that BD UAP tubes kept both urine chemistry and microscopy parameters stable up to 72 h. For microscopy analysis, more than 90% concordance was observed during the first 24 h, including all the parameters, and there was more than 80% concordance when the time interval was extended to 72 h; bacteria were the exception [7]. The components of BD UAP tubes are supposed to inhibit the metabolism of bacteria without causing morphologic changes; this is a favorable feature. In our study, bacteria were detected in three aliquots, two at 72 h and one at 24 h, while there were no bacteria detected at 0 h. In the 72-h aliquot, an increased pH was related to bacterial growth.

Lippi et al. suggested that tubes containing boric acid preserve strip results for 6–24 h with the exceptions of glucose and nitrite tests [8]. In our study, however, we found good concordance for all the chemical parameters in chlorhexidine-containing tubes even at 72 h.

Urine particle analysis should be performed as soon as possible after the collection of a fresh sample, whereas test strip urinalysis can be performed within 24 h of collection if the specimen is refrigerated [9]. Manoni et al. suggested that urine samples ($n = 300$) could be stored at room temperature up to 2 h without significant variation in the physicochemical and particle analysis results. With a longer storage period, they observed that the samples showed bacterial overgrowth and lysis in RBCs, WBCs, and casts [10]. In our study, samples in polystyrene tubes with no additives and refrigerated particle and chemistry analysis showed good agreement with the reference group with the exceptions of WBC clump and amorphous crystals at 8 h. False-positive WBC clump may lead to the misdiagnosis of a chronic infection, and increased amounts of amorphous crystals may prevent the accurate identification of other microscopic components in urine.

Urine storage for extended periods results in increased pH and may be due to urease producing *Proteus* sp.; particle lysis may also occur at higher pH values or at low specific gravity [11]. In our study, there was a significant change ($p = 0.042$) in pH only at 72 h compared to that at 0 h ($5.7 \pm 0.74$ vs. $5.5 \pm 0.52$, respectively). No significant difference in specific gravity ($p > 0.05$) was observed up to 72 h.

There were several limitations to this study. We did not perform manual microscopic examination, gold standard for particle analysis, which would be helpful for elucidating the possible changes in cell morphology. The study lacked a 48-h evaluation; which may be beneficial for reference laboratories and further studied. However 72-h was provided as the time point exceeding 24-h.

In conclusion, a chlorhexidine-containing preservative tube seems advantageous for the transport of urine samples from outside healthcare services to the Public Health Laboratory. When preservative tubes are used, keeping the samples at room temperature is recommended

because using ice packs produces a negative effect on WBCs and CaOx. If a delay in performing urinalysis is expected, using a preservative tube offers comparable results with refrigeration for the majority of parameters for 4 h and up to 8 h.

## Conflict of interest

None declared.

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