The effectiveness of BD Vacutainer® Plus Urinalysis Preservative Tubes in preservation of urine for chemical strip analysis and particle counting

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Abstract

Introduction: The aim of this study was to evaluate the stability of urine collected in preservative tubes for chemistry strip analyses and particle counting to determine whether the transport of urine samples with all of their constituents is possible.

Materials and methods: 275 pathologic urine specimens were included. Each urine sample was evaluated after 4, 8, 12, 24, and 48 hours of storage in BD Vacutainer® Plus Urinalysis Preservative (BD UAP) tubes and compared with refrigeration at 4 °C. All analyses were peformed on H-800 and FUS-200 automatic modular urine analyzers (Dirui Industry, Changchun, China). The kappa coefficients (κ), false positive (FP) and false negative (FN) rates were evaluated. $\kappa > 0.8$ was accepted as good agreement.

Results: Haemoglobin (Hb), leucocyte esterase (LE), and protein (Pro) analyses should be performed within 4 hours, whereas glucose (Glc) was stable until the end of 48 hours in both storage conditions. Nitrite (Nit) was well preserved in BD UAP tubes for 24 hours but was stable only up to 8 hours at 4 °C. Bilirubin (Bil) had very high FN rates even at 4 hours in both conditions. The particle counting showed high FN rates for white blood cells (WBC) and red blood cells (RBC), whereas squamous epithelial cells (EC) were stable up to 8 hours in both conditions.

Conclusions: Preanalytical requirements for both urine chemical strip analyses and particle counting in a unique sample were not met in either condition. Thus, the transfer of urine samples for centralization of urinalysis is not yet feasible.

Key words: urinalysis; preservation; sample preparation

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Introduction

Urinalysis is one of the most common analyses done in clinical laboratories (1). Automated urine analysers, including chemical strip readers and visual microscopic testing, have increased the efficiency of routine laboratories (2,3). However, despite improvements in the performance of analytic systems, the pre-analytical phase of modern urinalysis remains obscure (1). In both chemical strip analyses and particle counting, the sample must be kept stable until the analysis. The consolidation of laboratories has increased the physical distance between the patient and the laboratory, which creates a major pre-analytical challenge. Thus, it is necessary to focus on the pre-analytical phase to improve the reliability of test results (4) and lower the costs of health care (5). Solutions to the problem of stability of urine analytes will make the centralization of urine testing possible.

According to the Clinical and Laboratory Standards Institute (CLSI) guidelines (6), urine samples should be tested within 2 hours of collection. If a delay in analysis is inevitable, the samples must be stored under controlled conditions (i.e. temperature), and/or preservatives may be used for certain defined analyses (7). Refrigeration reduces cell degradation and bacterial growth, but crystallization of inorganic elements may affect the recognition of other particles in the sample (8). There is no agreed-on length of time for refrigeration for urine preservation because it depends on the individual

urine constituents (6). Preservatives have traditionally been used in many laboratories for specimens requiring microbiological investigation (9). Ethanol (50%) with or without polyethylene glycol (20 g/L) is used to preserve cellular particles (3,10). Containers supplemented with boric acid alone or in combination with formic acid are also used (3,11). Commercial preservatives, such as formaldehyde-based solutions, buffered boric acid, formate-based solutions and mercuric chloride–based tablets are also available (3). The CLSI recommends that, if commercially available urine preservation systems are used, these should first be evaluated in the laboratory. Such systems, which are useful for some analytes, may have some limitations for specific tests (6).

This study primarily aims to determine whether the transport of urine samples with all of their constituents collected in preservative tubes is feasible. Thus, we assessed urine stability for chemistry strip analyses (haemoglobin (Hb), leucocyte esterase (LE), nitrite (Nit), protein (Pro), glucose (Glc), bilirubin (Bil), urobilinogen (UBG), ketone (KET), specific gravity and pH) and particle counting (red blood cells (RBC), white blood cells (WBC), squamous epithelial cells (EC), and bacteria (Bact)) after 4, 8, 12, 24, and 48 hours of storage in a commercially available preservative tube in comparison to our in-home method of refrigeration. In evaluating the data, we used not only the usual concordance kappa coefficient (κ) but also false positive (FP) and false negative (FN) rates, which are of great clinical value.

Materials and methods

Subjects

This study was carried out at Dr. Lütfi Kırdar Kartal Training and Research Hospital from September to October 2014. The ethics committee of our institution approved the study, and the patients signed an informed consent to participate in the research. Patients from different policlinics who were referred to the laboratory for urinalysis were given urine cups with identification bar codes by the laboratory technician. The patients were instructed to collect an adequate volume (> 80 mL) of morning midstream urine in a clean plastic container and to bring the sample to the laboratory within 10 minutes after voiding. Our preliminary consideration was to reach a sufficient number of pathologic urine samples, especially for chemistry strip analyses and particle counting. Extremely mucoid, viscous or grossly bloody specimens were excluded; urine samples containing at least one pathologic analyte were included in the study.

Methods

Urine samples were filled into non-additive tubes, and analyses were done within 1 hour. Because we used non-additive tubes in our standard routine laboratory procedure (6), the results were accepted as the initial (reference) values. Each sample (8 mL) was aliquoted into five different non-additive tubes and five different preservative tubes, which were then inverted 8 to 10 times according to the manufacturer's instructions. BD Vacutainer® Plus Urinalysis Preservative (BD UAP) tubes (Ref no. 364992; Becton, Dickinson and Co., Franklin Lakes, NJ, USA) were used in the study. These are spray-coated polyethylene terephthalate plastic tubes (8 mL) containing 0.4% chlorhexidine, 5.6% ethylparaben, and 94% sodium propionate. BD Vacutainer® Urinalysis Plus conical urine tubes (Ref no. 364980; Becton, Dickinson and Co., Franklin Lakes, NJ, USA) of 8.0 mL draw volume and 16 × 100 mm size with no additive were also used. The preservative tubes were stored at room temperature (20 – 25 °C) and protected from light. The non-additive tubes were stored at 4 °C throughout the study. Taking the initial values as baseline, measurements were done at 4, 8, 12, 24, and 48 hours.

The analyses were carried out on H-800 and FUS-200 automatic modular urine analysers (Dirui Industry, Changchun, China). The H-800 urine chemistry analyser module is a reflectance photometer that measures the intensity of colorimetric changes in pad strips and converts them to categorical or semiquantitative data. Original multiparameter H-800 urine test strips were used (Dirui Industry, Changchun, China). The within-run percent coefficient of variations (%CV) in the urine analyses, cal-

culated from absorbance readings, were as follows: 2.9, 5.7, 8.7, 2.4, 4.4, 3.0, 7.1, 4.2 and 4.4 for Hb, Nit, LE, Pro, Glc, Bil, UBG, pH and specific gravity, respectively.

The FUS-200 urine sediment analyser module uses digital imaging technology. The automated classification was visually checked on the screen, and corrected if necessary by the same laboratory specialist. The within-run %CV obtained by the FUS-200 urine sediment analyser was 5.1 for an RBC count of (mean ± SD) 25.9 ± 1.3 x 10^6/L and 10.1 for a WBC count of (mean ± SD) 14.8 ± 1.5 x 10^6/L (12). An original urinalysis calibrator and control materials provided by the manufacturer (Dirui Industry, Changchun, China) were used. Daily calibration and quality control procedures were done according to the manufacturer's recommendations.

Statistical analysis

Statistical analyses were carried out by using MedCalc (version 15.4.0; MedCalc Software, Ostend, Belgium). For pH and specific gravity, the normality was evaluated by applying the Kolmogorov-Smirnov test; the Friedman test was used for nonparametric repeated measures. A $P < 0.05$ was accepted as significant. For Hb, LE, Pro, and Glc, the chemical strip results were expressed and classified as 1+, 2+, 3+, or 4+ in ordinal scale. For each time point (4, 8, 12, 24, and 48 hours), the number of urine samples in each category was compared with the initial counts as reference by using 4 x 4 contingency tables. The numbers of Nit- and Bil-positive urine samples were too small to express in 4 x 4 contingency tables. Considering that these analytes are undetectable in healthy urine and that the minimum reaction is pathologic, we categorized these samples qualitatively as either positive or negative.

For continuous scale measurements, such as for RBC, WBC, Bact, and EC, we used clinically important decision points: RBC > 15 x 10^6/L (13,14), WBC > 10 x 10^6/L (13-16), Bact > 150 x 10^6/L (13,14), and EC > 5 x 10^6/L (13), as cut off values and determined the number of pathologic samples in each category. Then we evaluated the changes at different time points, taking the initial counts as reference.

The agreement between results at different time points and the initial values was evaluated with the use of Cohen's kappa coefficients. A $\kappa > 0.80$ was accepted as good agreement, indicating that the analytes were stable. At each time point, the FP and FN rates were also calculated, taking the initial readings as reference.

Results

A total of 275 urine specimens were included in this study. Of these, 152 samples were obtained from female patients (55%), and 123 were from male patients (45%). The median age of the patients was 56 (range 8 - 85) years.

Table 1 shows the analytes, manufacturer-provided cut off values, and number of pathologic urine samples (%) for each parameter studied. Table 2 presents the initial number of urine specimens at each level (negative, trace, +1, +2, +3, and +4) of the ordinal scale.

Table 1. Manufacturer's cut-off values and number of pathologic urine samples

Analyte	Manufacturer's cut-off values	Pathologic urine samples, N (%)
Specific gravity (kg/L)	1.005–1.030	*
pH (pH units)	5.5–8.0	*
Nit (µmol/L)	Negative	40 (14.5)
Pro (g/L)	< 0.2	107 (38.9)
Glc (mmol/L)	< 5.6	73 (26.5)
Bil (µmol/L)	Negative	33 (12)
UBG (µmol/L)	Negative	4 (1.45)
KET(mmol/L)	Negative	4 (1.45)
Hb (RBC x 10^6/L)	< 10	155 (56.3)
LE (WBC x 10^6/L)	< 15	104 (37.8)
EC (x 10^6/L)	1–3	72 (26.1)

*All of 275 samples were included in statistical analysis.
Hb – haemoglobin, LE - leukocyte esterase, Nit – nitrite, Pro – protein, Glc – glucose, Bil – bilirubin, UBG – urobilinogen, KET – ketone, RBC – red blood cells, WBC – white blood cells. EC - squamous epithelial cells.

Table 2. Urine specimens by category

Analytes	Category				
	Negative / normal	Trace	+1	+2	+3
Hb	120	32	54	30	39
LE	171	40	38	13	13
Pro	168	40	32	31	4
Glc	202	-	18	10	45

Hb – haemoglobin, LE – leukocyte esterase, Pro – protein, Glc – glucose.

Chemical strip analyses

The initial (reference) pH value was (mean ± SD) 5.88 ± 0.40. After 48 hours storage the pH measured was 5.85 ± 0.45, with no significant change at 4 °C (P = 0.612). In BD UAP tubes, the pH value was 6.13 ± 0.26 at only the 4th hour (P < 0.001) and then did not show any more changes until the end of 48 hours (6.15 ± 0.39, P = 0.895).

The initial (reference) specific gravity was (mean ± SD) 1.015 ± 0.006 kg/L. At the end of 48 hours the specific gravity was 1.016 ± 0.006 (P = 0.776), with no significant change at 4 °C. In BD UAP tubes, the value changed to 1.017 ± 0.006 (P = 0.836) at the end of 48 hours, with no significant change either.

The number of Hb-positive samples decreased by 27% at 4 °C and by 36% in BD UAP tubes at the end of 48 hours. The number of positive leukocyte esterase samples decreased approximately by 23% both at 4 °C and in BD UAP tubes at the end of 48 hours.

Proteins showed the most discrepant results beginning from the 4th hour of storage. The κ values for storage at 4 °C were unacceptable and were even worse for specimens preserved in BD UAP tubes. The number of Pro-positive specimens decreased by 17% at 4 °C and by 20% in BD UAP tubes at only the 4th hour and then did not change anymore.

Glc was the most stable constituent. The number of Glc-positive samples was approximately the same throughout the 48 hours, with negligible changes.

A total of 235 Nit-negative and 40 Nit-positive urine specimens were evaluated. The number of Nit-negative specimens decreased by 13% at 4 °C and showed negligible change in BD UAP tubes at the end of 48 hours. In the BD UAP tubes, good concordance was achieved until the end of 24 hours, but at 4 °C it was stable only for 8 hours.

A total of 33 Bil-positive and 242 Bil-negative urine specimens were evaluated. The number of positive samples decreased by 33% at 4 °C and by 48% in BD UAP tubes at the end of 48 hours. There were only 5 urine samples with high UBG and 4 samples that were positive for KET. Thus, these parameters were excluded from the study.

Table 3 shows the agreement between the numbers obtained at 4, 8, 12, 24, and 48 hours and the initial (reference) values, as well as the FP and FN rates and the longest acceptable duration of preservation for chemical strip analyses at 4 °C and in BD UAP tubes.

Particle count analysis

There were initially 159 urine specimens below and 116 specimens above the cut off (15 x 10^6/L). The number of pathologic urine specimens decreased by 17% at 4 °C and by 42% in BD UAP tubes at the end of 48 hours. There were initially 167 urine specimens below and 108 specimens above the given cutoff (25 x 10^6/L). At 4 °C, the number of WBC-positive specimens decreased by 31% at 4 °C and by 65% at the end of 48 hours. Only 5 urine specimens had a Bact count > 150 x 10^6/L. Although this value was too small, the increase in the number of positive specimens at 4 °C was noteworthy, reaching 30 at the end of 48 hours. In the BD UAP tubes, 4 of 5 samples were preserved for 4, 8, and 12 hours, but none was detectable at the end of 24 hours. There were 72 urine specimens above and 203 specimens below the cutoff of 5 x 10^6/L. The values were approximately the same at the end of 48 hours, with a negligible change of 5%.

Table 4 shows the agreement between the numbers obtained at 4, 8, 12, 24, and 48 hours and the initial (reference) values, as well as the FP and FN rates and the longest acceptable duration of pres-

Table 3. Agreement of chemical strip analysis in initial referent samples with samples after storage

Analytes	Refrigeration				BD UAP tubes		
	Storage time (hours)	κ	FN (%)	FP (%)	κ	FN (%)	FP (%)
Hb	4	0.79	12.9	0	0.80*	12.9	2.2
	8	0.79	16.7	0.8	0.76	13.6	2.9
	12	0.75	18	2.5	0.78	15.1	2.9
	24	0.70	22.5	2.5	0.64	23	2.2
	48	0.64	32.2	5.8	0.58	30.2	2.2
LE	4	0.77	12.5	3.5	0.71	6.06	6.8
	8	0.72	20.1	2.9	0.74	9.09	5.11
	12	0.74	25.9	1.75	0.73	13.1	7.38
	24	0.76	18.2	4.67	0.72	17.1	9.68
	48	0.72	25.9	1.75	0.55	28.2	6.25
Pro	4	0.62	15.8	0	0.47	24.5	2.89
	8	0.62	16.8	1.19	0.52	18.6	1.73
	12	0.54	19.6	2.38	0.55	23.5	4.04
	24	0.54	20.5	2.9	0.44	21.5	4.04
	48	0.56	21.4	3.5	0.55	10.7	8.6
Glc	4	0.94	0	1.48	0.91	0	1.49
	8	0.87	1.36	1.48	0.89	1.35	0.49
	12	0.87	2.7	1	0.87	0	0.99
	24	0.88	4.1	2.47	0.88	0	2.98
	48	0.85*	2.7	1	0.80*	0	2.48
Nit	4	0.90	7.5	1.7	0.93	5.1	1.27
	8	0.83*	10	3.4	0.80	12.8	3.8
	12	0.71	27.5	3.4	0.82	10.2	3.8
	24	0.56	35	7.2	0.80*	10.2	4.6
	48	0.24	47.5	20.8	0.73	20.5	4.6
Bil	4	0.89	15.1	0.41	0.86*	20	0.4
	8	0.83*	21.2	0.82	0.76	36.7	0
	12	0.77	30.3	0.82	0.74	36.7	0.4
	24	0.70	39.3	0.82	0.68	43.3	0.4
	48	0.70	39.3	0.82	0.65	46.7	0.4

FN – false negative rates, FP – false positive rates, κ – kappa coefficient, Refrigeration – storage at 4 ˚C, BD UAP – storage in BD Vacutainer® Plus Urinalysis Preservative Tubes, Hb – haemoglobin, LE – leukocyte esterase, Pro – protein, Glc – glucose, Nit – nitrite, Bil – bilirubin.
*Longest acceptable stability duration for for chemical strip analyses at 4 ˚C and in BD UAP tubes (κ ≥ 0.80).

Table 4. Agreement of particle count analysis in referent samples with samples after storage

Analytes	Refrigeration				BD UAP tubes		
	Storage time (hours)	κ	FN (%)	FP (%)	κ	FN (%)	FP (%)
WBC	4	0.86*	11.1	3.8	0.65	35.2	3.6
	8	0.71	28.7	3.18	0.64	37.1	2.4
	12	0.65	34.2	3.8	0.61	41.9	1.8
	24	0.70	25	6.4	0.54	47.6	2.4
	48	0.49	44.4	8.9	0.27	72.3	3.63
RBC	4	0.86	14.6	3.78	0.78	29	7.3
	8	0.82*	22.4	4.54	0.79	33.3	10.6
	12	0.73	22.4	3.78	0.71	28.3	9.83
	24	0.67	16.3	6.06	0.63	31.2	9.01
	48	0.53	25	12.1	0.58	46.8	9.8
EC	4	0.88	4.16	4.9	0.86	8	4.5
	8	0.84*	16.6	1.97	0.81*	13.3	5.5
	12	0.75	20.8	5.4	0.77	14.6	7.5
	24	0.70	20.8	8.3	0.80	14.6	5
	48	0.68	26.3	6.8	0.81	17.3	3.5
Bact	4	0.60	10	3.7	0.66	40	0.3
	8	0.54	50	1.1	0.57	60	0
	12	0.59	40	1.5	0.28	80	0.3
	24	0.15	70	6.7	No result	No result	No result
	48	0.15	50	11.3	No result	No result	No result

FN – false negative rates, FP – false positive rates, κ – kappa coefficient, Refrigeration – storage at 4 ˚C, BD UAP – storage in BD Vacutainer® Plus Urinalysis Preservative Tubes, RBC – red blood cells, WBC – white blood cells, EC – squamous epithelial cells, Bact – bacteria.
*Longest acceptable stability duration for particle count analyses at 4 ˚C and in BD UAP tubes (κ ≥ 0.80).

ervation for particle counts at 4 °C and in BD UAP tubes.

Discussion

In the present study, different levels of agreement were achieved between the results obtained at different time points and the initial values for dif-

ferent parameters. Hb, LE, and Pro analyses should be done within 4 hours, whereas Glc was stable until the end of 48 hours in both conditions. Nit was well preserved in BD UAP tubes for 24 hours but was stable only up to 8 hours at 4 °C. Bil had very high FN rates even at 4 hours in both conditions. The particle counts showed high FN rates for WBC and RBC, whereas EC was stable only up to 8 hours in both conditions.

There were 155 Hb-positive urine samples, and we found that the agreement between the measurements at different time points and the reference values was not very good (κ values < 0.80) from the 4th hour onward, either at 4 °C or in BD UAP tubes. For the LE reaction, we evaluated 104 positive urine samples. The agreement between the results at different time points and the initial values was even worse than that for Hb in both storage conditions. In contrast, the agreement between the Pro results at different time points and the initial values all showed κ values < 0.62, and there was no gradual deterioration throughout the 48 hours. We found that the discrimination of ± 1 grade, especially at the negative/trace level, was poor. Because a certain amount of Pro is present in healthy urine, the measurement at this cut off level is a direct function of the strip performance. Bidirectional changes were observed mostly at this level, which caused low κ values, with false negatives being dominant. The Glc in urine seemed to be stable in both conditions until the end of 48 hours (κ values > 0.80). There were initially 40 Nit-positive urine specimens. At 4 °C, the number of Nit-positive specimens increased to 70 at the end of 48 hours. These were not all false positives. Bidirectional changes were observed when individual samples were evaluated. Bact growth in refrigerated urine tubes probably caused an increase in the Griess reaction for Nit detection (FP). However, once the analyte was produced, it could not be preserved for long hours (FN). At 4 °C, the agreement between the results and the initial values seemed to be acceptable up to 8 hours (κ = 0.83). The results obtained with the preservative tubes showed good agreement up to 24 hours (κ = 0.80), with a false negative rate of 10.2%.

Bil in urine seemed to be stable for 8 hours with refrigeration (κ = 0.83) and for 4 hours in BD UAP tubes (κ = 0.86). In both cases, the FN rates were over 20%. This indicates a conflict between the statistical result and the clinical outcome. Thus, if the Bil level in urine is of specific interest, it should be evaluated as soon as possible.

RBC and WBC were preserved for 8 and 4 hours, respectively, at 4 °C but only for 4 hours in BD UAP tubes. The FN rates were too high beginning from the first hours. Chemical preservatives that form an osmotic effect on cell membranes in combination with an alkaline pH cause rapid lysis of cells and morphologic changes. The pH values of urine samples in BD UAP tubes were more alkaline than those in non-preservative tubes at all sampling times. For the Bact evaluation, we only had a small number (5) of positive samples, not enough to run any statistical calculation. However, at 4 °C we observed Bact growth in 14 urine specimens immediately at the 4th hour.

Kouri *et al.* in their study on the preservation of urine-formed elements concluded that urine can be stored at room temperature up to 1 day (10). For longer periods of storage, they proposed the use of a preservative, especially to prevent artefactual bacterial growth. Kouri later studied urine preservation for both chemical strip analyses and particle counting and concluded that, if preserved properly, urine could be transported at 20 °C for both chemical strip analyses and particle counting. For storage periods longer than 72 hours, Kouri noted that refrigeration should be the preferred method (17). Eisinger *et al.* found the use of BD UAP tubes for microbiological testing to be an effective approach up to 48 hours; however, the authors did not apply chemical strip analyses (18). Miller analysed BD UAP tubes and found very good agreement between the results obtained at 24, 48, and 72 h and the initial values for all elements except Bact; this finding is different from our current results (19). The author analysed a small number of pathologic urine samples for each parameter (a total of 110 urine samples, 30% pathologic), but he calculated the agreements within ± 1 grade at 95% confidence level. Kappa values were also calculated, but these were not present-

ed in the study, probably because they were lower than the corresponding scores. The FN and FP rates were not given either.

In a recent study, Ercan *et al.* investigated the stability of urine specimens (48 samples) in the same BD UAP tubes stored at room temperature and on ice (20). Their study had a small sample size and a low prevalence of positive samples, and agreements were determined within ± 1 grade. As expected, the scores obtained in that study were very high compared with our κ-agreement values. The authors reported that chlorhexidine-based preservative tubes showed comparable results with refrigeration until 8 hours of storage, which is longer stability duration compared with our results. Further, they noted that refrigeration seemed to fail in the preservation of amorphous crystals and WBC clumps. However, the number of pathologic urine samples containing WBC clumps and amorphous crystals in their research were too small for any evaluation. In the present study, we did not observe an increase in turbidity in the urine specimens because of the precipitation of amorphous materials. Neither did the pH change in the course of the study.

The majority of studies on urine preservation and storage have focused on bacterial content and particle analysis for microbiological evaluation. Recently, the preservation of urine for chemical strip analyses to support the centralization of urine testing was investigated. These recent studies vary greatly in sample sizes, time points, instruments, and data analysis methods. Choosing the right method and deciding on the best presentation is very important in this regard considering that the mentioned calculations have led to different conclusions. In our opinion, the reason for the conflicting conclusions lies mostly in the interpretation of data.

The present study evaluated 275 urine samples, all containing at least one analyte at the pathologic level. Our sample size of pathologic urine is rather large, and we adopted more time points and different methods of data evaluation compared with previous reports in the literature. We did not include analyte/particle-negative urine samples in the relevant calculations of κ because κ-agreement statistics is very sensitive to prevalence. For instance, in the calculations for Hb, we only included 155 Hb-positive urine samples. If we had included the whole sample, the final κ values would be greater because of agreement with the large number of analyte-negative urine. We categorized the semiquantitative results for Glc, Hb, LE, and Pro into either positive or negative and provided the FN/FP rates, which are of great clinical importance. During the preservation period, both increases and decreases in analytes and particle counts may occur, and the final number of positive/negative samples may be regarded as unchanged. These bidirectional changes are clinically important and should be emphasized. On the other hand, calculations accepting results within ± 1 grading difference as partial agreement always show higher agreement compared with κ values. As with κ statistics, taking negative samples into consideration manipulates the agreement scores. In contingency tables used for symmetry evaluation, false negatives and/or false positives, which have great clinical importance, do not draw specific attention. When contingency tables are used to show the agreement of results between different time points (not between raters), FN/FP rates become more important than the symmetry around the diagonal line. Alternatively, overall agreement is another calculation used to compare two raters. κ statistics is affected by the marginal totals, whereas overall agreement is not. Therefore, data should always be presented with "percent positive / negative agreement" values or FP/FN rates, and the clinical decision points must be interpreted carefully (21). Kouri *et al*. used failure rates in their evaluation and proposed a failure rate of 10% or less as acceptable (17). Another important point is that κ values may be evaluated with different cut offs by different authors. A value > 0.80 is accepted as very good agreement, 0.60 - 0.80 as good, and 0.40 - 0.60 as moderate. Generally, authors in medical research regard $\kappa > 0.80$ as good agreement and $\kappa < 0.60$ as unacceptable (22). Low levels of inter-rater reliability are not acceptable in health care or clinical research, especially when the results may lead to poorer patient outcomes.

In the current study, besides using the κ coefficient to indicate agreement between the results, the observed differences at different time points were evaluated with special emphasis on their contribution to clinical interpretation.

One limitation of this study is that we did not carry out manual microscopic examination, which is the gold standard for particle counting. Also, the particle counting results were checked by the same laboratory specialist as recommended. This may be considered as a limitation of the technique itself.

In conclusion this study was primarily done to evaluate the stability of urine for chemical strip analyses (Hb, LE, Nit, Pro, Glc, Bil, UBG, and KET levels; specific gravity and pH; and particle counts (RBC, WBC, EC, and Bact)) at 4, 8, 12, 24, and 48 hours of storage in a commercially available preservative tube and to compare the results with our in-home method of refrigeration. The observed differences at different time points were evaluated with special emphasis on their contribution to clinical interpretation.

Hb, LE, and Pro analyses should be done within 4 hours, whereas Glc was stable until the end of 48 hours in both conditions. Nit was well preserved in BD UAP tubes for 24 hours but was stable only up to 8 hours at 4 °C. Bil had very high FN rates even at 4 hours in both conditions. The particle counts showed high FN rates for WBC and RBC, whereas EC was stable up to 8 hours in both conditions.

These results indicate that the transfer of urine samples for centralization of urine testing is not yet feasible. In both conditions, the pre-analytic requirements of both chemical strip testing and particle counting in a unique sample were not met. Besides, a newly introduced preservative should be evaluated in the laboratory, paying special attention to the FP and FN rates, which are very important from the clinical point of view.

Potential conflict of interest

None declared.

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