Evaluation of alternative approaches to the reference method for the urine osmolality test

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ABSTRACT

Introduction: urine osmolality, a crucial test for differential diagnosis of hydro-electrolytic disorders, is mainly assessed by measuring the colligative properties of solutes. Many approaches were attempted to assess urine osmolality without this reference technique, mainly based on the use of formulas. In this study the performance of the Sysmex UF-4000/5000 system, which provides an alternative approach for estimating urine osmolality by means of conductivity, has been evaluated.
Methods: the Passing-Bablok regression and Bland-Altman plots have been used to compare the results obtained by the reference method and calculated with seven different forlmulas from 122 routine urine samples.
Results: none of the formulas demonstrated acceptable performances. Sysmex-calculated osmolality showed no systematic error and a bias to the reference method lower than the minimum acceptable as calculated according to biological variation data. To evaluate a possible effect of glucose on colligative properties, we also used a subset of samples with glucose ≤5.6 mmol/L (100 mg/dL); in these samples, the Sysmex-calculated osmolality and one of the formulas - 1.09 [1.86 Na (mmol/L) + glucose (mmol/L) + urea (mmol/L)] - showed a variation lower than desirable bias.
Discussion: the Sysmex UF-4000 analyzer demonstrated a good agreement with the reference technique, but a significant bias was still present. The seven different formulas tested in our investigation showed even lower analytical performances, so that the use of Sysmex analyzer may be preferable in facilities where osmometers are unavailable.

Key words: *osmolality, electric conductivity, urinalysis*

INTRODUCTION

Urine osmolality is an important test for the differential diagnosis of hydro-electrolytic disorders. Urine is constituted for up to 97-99% of water, while the remaining 1-3% is a mixture of solutes, the concentration of which is an important marker of renal function (i.e. ability to concentrate the urine) and hydration status. The expression of solutes in urine can be assessed using several parameters that vary in significance and type according to the solutes being measured, and include relative density, conductivity, and osmolality (each of these parameters can be measured with various methods) (1).

The relative density expresses the density of urine in mass/volume (typically in g/L), and is the most common test used in clinical practice (2). The conductivity depends on the concentration of the electrolytes in urine (sodium, chloride, potassium and even ammonia), with little impact of uncharged molecules such as glucose and proteins.

Osmolality is determined using methods that measure the colligative property of a solution, which depends solely on the number of particles dissolved, without impact of particle size, density, configuration, or electrical charge. Osmolality (Osmol/kg H_2O) is a more thermodynamically accurate expression than osmolarity (Osmol/L of solution) because solute concentrations normalized for the body weight are temperature-independent, whereas volume-based concentrations may vary with temperature. The reference method for osmolality is based on osmometers,

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which assess the lowering of the freezing point of the test solution in comparison with water (3). However, this type of instrumentation is scarcely prone to automation, making it almost unsuitable for emergency medicine applications; it may even be unavailable in all clinical laboratories.

Many approaches were hence attempted to measure osmolality in the absence of the reference method, the most common of which is the use of specific formulas, mostly including the values of urine sodium, glucose, and urea measured on automated clinical chemistry platforms (4).

The Sysmex UF-4000 and UF-5000 instrument (Sysmex Corp, Kobe, Japan) has been recently introduced into the market, allowing automated assessment of urine conductivity and better adaptation to routine use. In addition, this instrument uses a proprietary algorithm to estimate urine osmolality based on conductivity. The goal of our study was to assess osmolality using different formulas and the Sysmex analyzer, and then compare the results with the reference method.

METHODS

122 non-consecutive urinary samples, with a routine request for urinalysis have been collected over the course of two weeks. In all the samples, a complete standard urinalysis (as requested) using a Sysmex UN-series modular system (Sysmex Corporation, Kobe, Japan), was performed; the test includes a Sysmex UC-3500 (automated chemistry analyzer), a MediTape UC-11A test strips, and a Sysmex UF-4000 for particles examination. Moreover, within one hour after urinalysis was completed, an osmolality test using the reference method (i.e., by means of an osmometer: Osmo Station OM-6060; Arkray Inc., Kyoto, Japan) was performed and sodium (Na), urea, and glucose on a Roche Cobas c502 using proprietary reagents (Roche Diagnostics, Rotkreuz, Switzerland) were measured.

The results of measurements using Roche Cobas c502 were then used to calculate urine osmolality with seven different formulas (4), as follows:

- Formula 1 (then referred as to F1) (5): 2 Na (mmol/L) + glucose (mmol/L) + urea (mmol/L);
- Formula 2 (then referred as to F2) (6): 1.86 Na (mmol/L) + glucose (mmol/L) + urea (mmol/L) + 9;
- Formula 3 (then referred as to F3) (4): 1.09 [1.86 Na (mmol/L) + glucose (mmol/L) + urea (mmol/L)];
- Formula 4 (then referred as to F4) (7): 1.86 Na (mmol/L) + glucose (mmol/L) + urea (mmol/L);
- Formula 5 (then referred as to F5) (8,9): 1.86 Na (mmol/L) + glucose (mmol/L) + urea (mmol/L) + 5;

Formula 6 (then referred as to F6) (10): 2 Na (mmol/L) + glucose (mg/dL)/ 20 + urea (mg/dL)/6.4;

- Formula 7 (then referred as to F7) (11): 1.75 Na (mmol/L) + glucose (mmol/L) + urea (mmol/L) + 10.1.

The results obtained with the Sysmex analyzer and those calculated with the seven different formulas were tabulated in a Microsoft Excel 365 file (Microsoft Corp, Redmon, WA, USA) and then analyzed with Pearson's correlation, Passing-Bablok regression and Bland-Altman plots using Vidali MetComp 1.0 (12).

To account for the possible interference of high glucose concentrations in calculating osmolality, the same statistical analysis was carried out in a subset of samples showing glucose ≤5.6 mmol/L (100 mg/dL) as measured by an automated dipstick with Sysmex UC-3500 (part of the Sysmex UN urinalysis system).

RESULTS

The urine osmolality of the 122 samples measured with the reference method ranged between 185 and 1166 mOsm/kg H_2O (mean 583 mOsm/kg H_2O, SD 213, median 568). Of these, 105 samples had glucose values ≤5.5 mmol/L (100 mg/dL) and a corresponding urine osmolality comprised between 185 and 1166 mOsm/kg H_2O (mean 575, SD 221, median 554). For all the statistical analyses the results obtained with the osmometer were considered as the reference method, and Sysmex or formula results as the methods under investigation.

The results of Pearson's correlation with the reference method are summarized in Table 1, ranging 0.728-0.821 by Sysmex and between 0.906-0.972 by the different formulas. F2 yielded the best overall correlation to the reference method.

The results of the Passing-Bablok regression and Bland-Altman plot analysis are shown in Table 2 for all the 122 samples, and in Table 3 for the 105 samples with low-to-zero glucose levels. Since in all comparisons with the reference method the bias was statistically significant, the percent bias was also compared (Table 2 and 3) with the desirable bias calculated from biological variation, using the threshold defined for morning urines by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) biological variation database (13, 14), which reports a within (CV_I) and between (CV_G) subject variation of 28.3% and 57.9%, respectively. The desirable maximum bias calculated with the formula (15)

$$k \times \sqrt{(CV_I^2 + CV_G^2)}$$

Table 1
Pearson test results compared with the reference method.

	122 samples	105-samples subset with low-to-zero glucose levels
Sysmex	0.728	0.821
F1	0.934	0.962
F2	0.951	0.972
F3	0.939	0.966
F4	0.939	0.966
F5	0.939	0.966
F6	0.906	0.959
F7	0.941	0.968

F1 to F7, the seven formulas

Table 2

Passing-Bablok regression and Bland-Altman plot analysis for all the 122 urine samples

	Passing-Bablok regression					Bland-Altman analysis				Acceptable bias using biological variation data
	Slope	(95%CI)	Intercept	(95%IC)	MetComp comment	Bias %	(95%IC)	95% interval of differences (as %)	MetComp comment	
Sysmex	0.92	0.80-1.07	-25.40	-110.39 to 43.43	No systematic error	17.80	12.28- 23.32	-42.56 to 78.17	Significative bias	Better than minimum
F1	0.83	0.78 - 0.87	-9.23	-30.34 to 20.3700	Proportional systematic error	21.87	19.53- 24.22	-3.78 to 47.52	Significative bias	Better than minimum
F2	0.68	0.64 - 0.71	-7.79	-28.61 to 10.85	Proportional systematic error	41.86	39.94- 43.78	20.87 - 62.85	Significative bias	Not acceptable
F3	0.88	0.83 - 0.93	-10.21	-34.77 to 19.87	Proportional systematic error	16.31	14.05- 18.58	-8.47 to 41.10	Significative bias	Better than minimum
F4	0.80	0.76 - 0.85	-8.40	-31.23 to 19.17	Proportional systematic error	24.81	22.57- 27.05	0.31 - 49.31	Significative bias	Not acceptable
F5	0.80	0.76 - 0.84	-3.40	-26.23 to 24.17	Proportional systematic error	23.57	21.33- 25.80	-0.83 - 47.96	Significative bias	Better than minimum
F6	0.80	0.75 - 0.85	-11.89	-36.07 to 16.88	Proportional systematic error	27.00	24.15- 29.84	-4.11 - 58.10	Significative bias	Not acceptable
F7	0.79	0.75 - 0.83	0.91	-17.65 to 24.88	Proportional systematic error	24.63	22.48- 26.78	1.16 - 48.10	Significative bias	Not acceptable

Table 3
Passing-Bablok regression and Bland-Altman plot analysis for the 105 urine samples with low-to-zero glucose levels

	Passing-Bablok regression					Bland-Altman analysis				Acceptable bias using biological variation data
	Slope	(95% C.I.)	Intercept	(95%IC)	MetComp comment	Bias %	(95%IC)	95% interval of differences (as %)	MetComp commentp	
Sysmex	0.91	0.78 - 1.05	2.314	-77.66 to 55.06	No systematic error	12.55	7.62 - 17.49	-37.43 to 62.53	Significative bias	Better than desirable
F1	0.84	0.79 - 0.89	-6.41	-31.14 to 17.04	Proportional systematic error	20.44	18.24 - 22.64	-1.85 to 42.73	Significative bias	Better than minimum
F2	0.69	0.65 - 0.72	-13.50	-34.12 to 5.33	Proportional systematic error	41.16	39.32 - 43.00	22.55 - 59.77	Significative bias	Not acceptable
F3	0.89	0.84 - 0.94	-7.03	-32.34 to 16.26	Proportional systematic error	14.96	12.85 - 17.07	-6.42 to 36.35	Significative bias	Better than desirable
F4	0.81	0.77 - 0.86	-6.01	-29.02 to 15.25	Proportional systematic error	23.47	21.38 - 25.56	2.29 - 44.65	Significative bias	Better than minimum
F5	0.81	0.77 - 0.86	-1.01	-24.02 to 20.25	Proportional systematic error	22.21	20.13 - 24.28	1.20 - 43.22	Significative bias	Better than minimum
F6	0.81	0.76 - 0.85	-1.92	-31.22 to 16.63	Proportional systematic error	24.02	21.72 - 26.31	0.77 - 47.27	Significative bias	Better than minimum
F7	0.80	0.76- 0.84	0.53	-17.01 to 21.69	Proportional systematic error	23.32	21.35 - 25.31	3.25 - 43.40	Significative bias	Better than minimum

yielded to a minimum allowable bias (k=0.375) of 24.2%, a desirable (k=0.25) bias of 16.1% and an optimal bias (k=0.125) of 8.1%. In the entire set of urine samples, the bias was better than minimum using Sysmex, and three formulas, while it was unacceptable with all other equations. In the 105 samples with low-to-zero glucose levels the bias was better than desirable using Sysmex and with F3, while better than minimum with F1, F4, F5, F6 and F7. Across all comparisons with the reference method and type of samples, the values obtained with Sysmex displayed the best slope and lowest percentage bias

Passing-Bablock and Bland-Altman plots for Sysmex and F3 formula versus reference method are shown in Figure 1.

DISCUSSION

The assessment of urine osmolality is important in clinical practice as index of urine concentration. The reference method for this measurement is at the moment the determination of the freezing point depression of the test solution compared to water using an osmometer. However, this method suffers from several practical limitations, as mentioned above.

Searching on PubMed "Sysmex AND osmolality" (searched on April 9th, 2024) 8 reports have been retrieved, but only 3 of these analyzed the correlation between osmolality calculated from conductivity (using Sysmex UF-4000 or UF-5000[1)].

Oyaert et al. (16) tried to improve the manufacturer's algorithm for the Sysmex UF-5000 adding the standard urinalysis parameters such as creatinine, glucose, and relative density. They tested different correlation patterns, and observed that a multiple mixed approach (multiple linear mixed model analysis) revealed that the strongest predictors of measured osmolality were electrical conductivity, compounded by creatinine and relative density. This resulted in a correlation coefficient (r^2) value of 0.895 in the very limited set of samples they analyzed (n=36 patients).

Yoo et al. (17) validated the UF-5000 by comparing urine osmolality obtained with OsmoPro osmometer (I&L Biosystems GmbH, Königswinter, Germany). The results obtained with UF-5000 showed acceptable linearity over some of the evaluated concentrations. The overall results of the comparison study suggested that the conductivity-based measurement of osmolality had reliable precision and linearity within a specific scale of values within the reference range. This persuaded the authors to conclude that UF-5000 can be used to determine whether urine osmolality is within the reference range, otherwise it should be assayed using the reference technique.

Yis et al. (18) also designed a study to evaluate the performance of urine osmolality with UF-5000, and to specifically investigate the effect of the presence of different molecules and particles. The authors performed a complete urinalysis and conductivity-based osmolality analysis with UF-5000, and with a 3320 Micro-Osmometer (Advanced Instruments LLC, Norwood, MA, USA). Samples were classified as negative, or either positive for glucosuria, proteinuria, hematuria, pyuria, crystalluria, and urobilinogen. The results confirmed what was previously observed by Yoo et al. (17). In fact, considering its optimal accessibility, it could be concluded that UF-5000 can be considered a reliable option to determine whether urine osmolality is within the reference range, otherwise methods based on colligative properties should be used.

In the present study, the osmolality calculated by Sysmex performed acceptably, with no systematic error, obtaining better results than all other formulas, with a statistically acceptable slope and intercept resulting from the Passing-Bablok regression. However, concerning confidence intervals, the intercept derived from the regression analysis appears higher than those of the formulas.

In terms of bias, the values obtained with this analyzer had an acceptable minimum level for the totality of urine samples and also reached a desirable level for those with low glucose. These results confirm the possibility of using the Sysmex as an alternative to the osmometer for determination of urine osmolality, taking into account glucose levels measured with dipsticks to better inform on possible bias.

Bianchi et al. colleagues carried out a study with a different purpose than assessing Sysmex UF-4000/5000 performance (4), i.e., to evaluate whether the calculated osmolality could vicariate direct measurement in both serum and urine. They used the same seven formulas as in our study to calculate osmolality in 116 plasma and 94 urine samples; the results were then compared with those of the reference method. With the exception of some formulas, the calculated osmolality was always lower than the measured osmolality for both plasma and urine. This result can be explained by the fact that many analytes contribute to the osmolality of biological fluids. The correlations between calculated and measured osmolality were poor for plasma and better for urine, regardless of the formula used. Therefore, the authors concluded that despite various mathematical formulas in the scientific literature, direct measurement of osmolality is always recommended to obtain a better assessment of patient status.

Concerning the results of our investigation, we can confirm that the seven tested formulas cannot be considered an excellent alternative for measuring urine osmolality. All the formulas that performed worse than the conductivity-based calculation, included glucose. However, usually only sodium and urea are included in the final calculation (as representative ions) as conductivity is much more meaningful as it evaluates all charged molecules as a whole (e.g. the presence of potassium, chlorine, magnesium, calcium and other ions dissolved in the solution). The F3 formula, although evidencing a statistically significant systematic error, can be considered the best among the formulas used, as the

[1]Sysmex UF-4000 and Sysmex UF-5000 differ only for the sample processing, that is much more rapid for UF-5000.

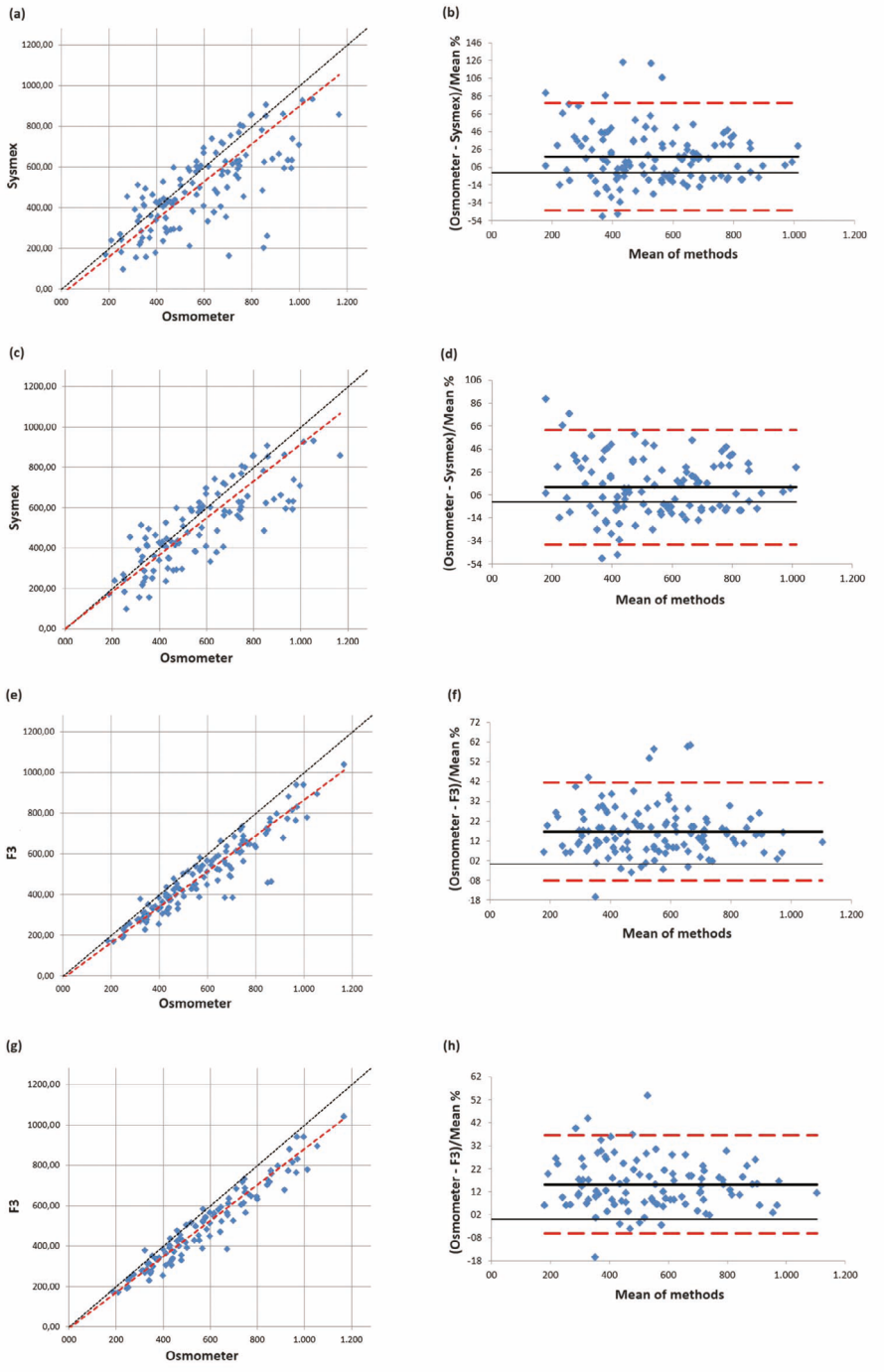

Figure 1
Passing-Bablock regression (panel a) and Bland & Altman graphs (panel b) graphs for Sysmex and for F3 formula. All results are expressed in mOsm/kg H_2O
(a) Sysmex versus Osmometer Passing-Bablok in all samples
(b) Sysmex versus Osmometer Bland-Altman in all samples
(c) Sysmex versus Osmometer Passing-Bablok in low-glucose sample group
(d) Sysmex versus Osmometer Bland-Altman in low-glucose sample group
(e) F3 versus Osmometer Passing-Bablok in all samples
(f) F3 versus Osmometer Bland-Altman in all samples
(g) F3 versus Osmometer Passing-Bablok in low-glucose sample group

slopes of the Passing-Bablok regression were the best among the different formulas (comparable to Sysmex's data). In addition, the bias obtained by Bland-Altman analysis was as good as the one obtained with Sysmex in all-samples including the low-glucose groups, with narrower confidence intervals and interval of differences.

A limitation of the study is that the clinical utility of Sysmex osmolality calculated was not assessed in particular contexts (i.e., patients in particular conditions with excretion of solutes with osmotic activity, such as mannitol or other metabolites related to pathologic conditions), so that additional studies will be needed to better address how test results could be modified in these conditions.

In conclusion, the new Sysmex analyzer showed an acceptable correlation with the reference technique, but still presented a significant bias in both the all-samples group and the low-glucose group. However, for samples with glucose ≤5.6 mmol/L (100 mg/dL), such bias was below the desirable bias limit calculated from the biological variation, thus making the Sysmex UF-4000 an acceptable alternative to the reference technique for measuring urine osmolality in samples with low glucose concentration, especially when the reference method is not available. Actually, the seven formulas tested had lower analytical performances, so the use of the Sysmex analyzer may be preferable when an osmometer is unavailable.

CONFLICT OF INTEREST

None

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