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Evaluation of conductivity-based osmolality measurement in urine using the Sysmex UF5000

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Abstract

Background: Automated flow cytometry-based urine analyzer is increasingly being used to identify and enumerate cells and particles in urine specimens. It measures electrical conductivity which could be transformed to osmolality. Using this machine, all urine specimens could be screened for osmolality without requiring a separate dedicated device. We evaluated the performance of the new instrument, the UF-5000 (Sysmex Corporation), in the measurement of urine osmolality.

Methods: The precision of urine osmolality measurement by the UF-5000 was evaluated for 20 days and 4 times a day for 2 concentrations. The linearity and detection capability were evaluated according to the Clinical and Laboratory Standards Institute guidelines. For comparison, 270 random urine specimens from patients were tested simultaneously using the UF5000 and the OsmoPro micro-osmometer (Advanced instruments).

Results: The laboratory-based coefficient variations were less than 5%. Urine osmolality using the UF-5000 has a verified linear range ($y = 1.097x + 16.91$, $R^2 = .997$). Within the comparison analysis, the mean difference was not large (−7.72%) but each differences were largely dispersed with 95% limits of agreement (LoA) from −70.5 to 55.06%, and the mean absolute difference −28.3 mOsm/kg with 95% LoA from −295.13 to 238.45 mOsm/kg. Cohen's kappa value was 0.54 (95% CI, 0.45-0.63).

Conclusions: The UF-5000 measured conductivity and generated an acceptable quantitative analysis of urine osmolality. When compared with the results of the freezing point depression method used by the OsmoPro, a percentage of the measured urine osmolality by the UF-5000 was outside the allowable limit.

KEYWORDS
automated urine analyzer, comparison, conductivity, OsmoPro, UF5000, urine osmolality

1 | INTRODUCTION

Urinalysis is a convenient and non-invasive screening tool for use in both an outpatient and inpatient setting.[1,2] Despite its simplicity, urinalysis provides valuable information, such as urine acidity (pH) and specific gravity (SG), as well as the presence of protein, glucose, red blood cells (RBCs), white blood cells (WBCs), and bilirubin. Abnormal findings in routine urinalysis may be the first indication of a disorder involving the kidneys and urinary tract, even in asymptomatic patients. Urinalysis can also be used to monitor the progression of kidney disease.

The osmolality of urine, which indicates the quantity of osmotically active particles, can be estimated by the SG. While the SG correlates with urine osmolality, the direct measurement of urine osmolality is more accurate and is considered the gold standard for determining the hydration status of the body and its ability to concentrate urine. Low urine osmolality may occur with increased water intake, vasopressin deficiency, or diabetes mellitus. High urine osmolality is common in hypovolemic states, such as dehydration, that cause reduced renal blood flow and damage to renal tubular cells, which thus impairs the urine-concentrating ability of the kidneys.[3]

The extent to which the urine can be cooled to below 0°C is depended on concentration of dissolved substances.[4] This is referred to as the freezing point depression method, which is widely used due to its superiority, precision, and accuracy. However, a separate device is required, which impacts its practicality. Further to this, its accuracy can be lowered when cloudy specimens are used as the particulate matter causes precipitation in the sample during freezing and thus can interrupt the measurement cycle.

Urine osmolality can also be estimated using electrical conductivity. This method has been used since the beginning of the last century for different applications.[1,5] Electrical conductivity, or resistivity, estimates the total amount of ions present in biological fluids such as urine. The ionic concentration of urine determines the ability of the fluid to conduct an electrical current in a non-linear function. This method is non-selective for each molecule, but it is highly sensitive, user-friendly, and measures conductivity without the addition of chemical reagents. The UF-5000 from Sysmex Corporation, Japan, is an example of an instrument that measures urine osmolality via the principle of electrical conductivity.

In our study, we compared urine osmolality obtained using the OsmoPro, which uses freezing point depression, and the UF-5000 analyzer, which uses electrical conductivity. This was to determine whether urine osmolality can be reliably measured using the common urine sediment analyzer without requiring the use of a separate osmometer.

2 | MATERIALS AND METHODS

2.1 | Samples

Various samples were used for the evaluation of osmolality by the UF-5000 equipment (Sysmex Corporation). Commercial control materials with low and high osmolality were used for the evaluation of precision. Limit of blank (LOB) and limit of detection (LOD) measurements were performed with distilled water, as well as quality control (QC) and low osmolar urine samples. The linearity evaluation was also performed by mixing materials with low and high osmolar urine samples remnant after clinical tests using the OsmoPro analyzer (Advanced Instruments). A comparison analysis between the UF-5000 and the OsmoPro equipment was performed in the laboratory using random urine samples from patients.

Random urine samples, also known as spot samples, were collected for analysis that included osmolality testing from May 2018 to July 2018 in Pusan National University Yangsan Hospital. The samples with sufficient volume, which consisted of more than 5 mL, were selected for our study. The osmolality test required urine from healthy participants; subsequently, 270 urine samples that did not generate abnormal results from patients among those who visited the health examination center were used in this study. Residual urine samples were stored for one day and discarded according to the laws and regulations for the storage and disposal of human derivatives. This study was approved by the Institutional Review Board of our hospital (No. 05-2018-134). The use of residual urine samples to evaluate the test method was considered, and thus, the requirement to obtain participant consent was waived.

2.2 | Precision

Precision was evaluated according to the Clinical and Laboratory Standards Institute (CLSI), specifically the evaluation of precision of quantitative measurement procedure approved guideline 3rd edition (EP05-A3).[6] The commercial quality control (QC) material Liquichek Urinalysis Control (Bio-Rad Laboratories Inc.) with two concentrations, 430 and 767 mOsm/kg, was used. For 20 days, the assay was performed four times a day (twice in the morning and twice in the afternoon) and each of the control samples with known concentrations was evaluated by alternating the order of analysis. In addition to the quality control material, patient samples were measured 5 times for a period of 4 days to determine instrument precision. The samples measured by the UF-5000 within approximately 10% of the lower reference range were selected.

2.3 | LOB and LOD

The LOB and LOD were set with reference to the CLSI evaluation of precision of quantitative measurement procedure approved guideline 2nd edition (EP17-A2) document.[7] The LOB and LOD were confirmed by repeated analysis of the QC material, clinical samples, and distilled water. We evaluated the two reagents Lots (Lot A and Lot B) separately, as we anticipated there to be no differences in the results due to the differences in Lot numbers. For the LOB, 4 volumes of distilled water were repeated 5 times daily for 3 days, resulting in 60 measurements. The LOD was evaluated using low osmolar residual

TABLE 1 Precision test results of osmolality measurement using UF-5000

Variable (Unit)	Level	Mean (SD)	Manufacture value	Relative bias	SD (% CV)	
					Within day	Within laboratory
QC material (mOsm/kg)	High	636.8 (3.77)	430	17%	29.1 (4.6%)	30.2 (4.7%)
	Low	404.7 (1.83)	767	5.9%	16.2 (4.0%)	16.2 (4.0%)
Patient sample (mOsm/kg)	Low	317.6 (3.04)	NA	NA	10.3 (3.2%)	11.0 (3.5%)

Abbreviations: CV, Coefficient of variation; NA, Not applicable; SD, Standard deviation.

urine samples via the same protocol as LOB. The mean and standard deviation (SD) were obtained from the measured values, and the LOB and LOD were set according to the following CLSI guideline EP17-A2:

$$LOD = LOB + 1.645 \times (SD_{low\ sample}).$$

2.4 | Linearity

Linearity was tested and evaluated with reference to the CLSI EP06-A.[8] The linearity of urine osmolality was evaluated by dilution analysis using low (L) and high (H) osmolar remnant urine. H and L samples were mixed, and five evenly distributed materials were prepared as follows 1: L, 2:0.875L + 0.125H, 3:0.750L + 0.250H, 4:0.625L + 0.375H, and 5:0.500L + 0.500H. The osmolality of the diluted samples was calculated manually and then measured by UF-5000. We used regression analysis to calculate first, second, and third order polynomials. The linearity was evaluated through the allowable limit between the linear regression equation and the most suitable non-linear curve. The allowable limit was determined as half of the 30 mOsm/kg, which is 10% of the lower limit of the reference value.

2.5 | Methods comparison

The comparison was done according to the CLSI Measurement Procedure Comparison and Bias Estimation Using Patient Samples (EP09-A3) document.[9] Between 21 May and 23 July 2018, we randomly collected daily urine specimens from patients as described above in Section 2.1. Urine osmolality was measured by using the OsmoPro (Advanced instruments, Norwood, MA, USA) and then the UF-5000. Each urine specimen was tested using both methods, which were performed within 10 minutes.

2.6 | Statistical method

Data were analyzed using the software Analyze-it Method Evaluation Edition, version 3.76 (Analyze-it Software Ltd.), EP evaluator release 12.0 software (David G. Rhoads Associates), and IBM SPSS Statistics 22 (International Business Machines Corp.). Pearson's correlation coefficients and Passing-Bablok linear regression equations were used for comparison analysis between the osmolality readings from the OsmoPro and UF-5000. The absolute and relative differences between the two analyzers were presented in a Bland-Altman plot,
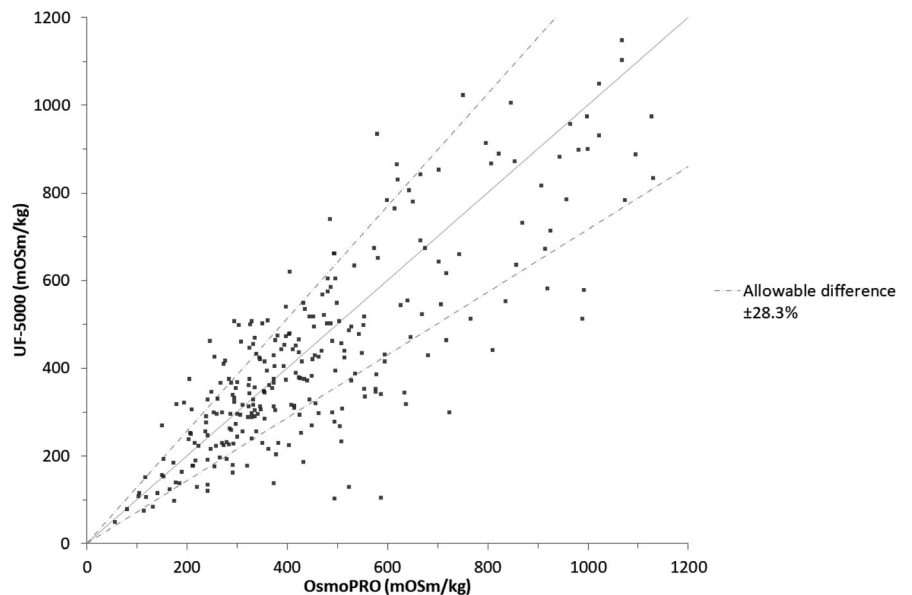
FIGURE 1 Passing-Bablok regression plot comparing UF-5000 to OsmoPro osmometer. The continuous line represents the equality line, and the dotted lines represent allowable difference ± 28.3% interval

TABLE 2 Compared linearity between polynomial fit and linear fit (Unit: mOsm/kg except %)

Dilution ratio	Assigned value	Mean	Polynominal Fit	Line Fit	Deviation from Linearity	Deviation Percent (%)
Low	21.5	21.5	25.71	40.49	−14.79	−36.5%
7:1	416.7	491.0	481.37	474.01	7.36	1.6%
6:2	815.0	922.0	925.75	910.93	14.82	1.6%
5:3	1211.8	1349.0	1353.61	1346.20	7.41	0.6%
4:4	1608.5	1769.5	1766.57	1781.36	−14.80	−0.8%

while the OsmoPro results were plotted on the x-axis and the UF-5000 results were plotted on the y-axis. Precision assessment was done by obtaining the mean, SD, and coefficient of variation (CV). P values <.05 were considered to be statistically significant. The diagnostic performance was compared between the OsmoPro and UF-5000 analyzers by measuring kappa coefficients with 95% confidence intervals (CI).

3 | RESULTS

3.1 | Precision

The mean (SD) urine osmolality was 636.8 (3.77) mOsm/kg for the high and 404.7 (1.83) mOsm/kg for the low osmolality samples. Table 1 shows the precision test results of osmolality evaluated by the UF-5000. The CV values within the laboratory were 4.7% and 4.0% for the low and high osmolality samples, respectively, and were within 5%. There was no significant difference between precision when measuring high and low osmolality. The bias between the measured value and the value assigned by the manufacture was 17% for the high and 5.9% for the low control. In the evaluation of the precision using patient samples, the mean osmolality was 317.6 (3.04) mOsm/kg, and so within the laboratory, precision was 3.5%.

3.2 | LOB and LOD

The LOB data measured in distilled water were 15.83 mOsm/kg and 33.29 mOsm/kg for Lot A and Lot B, respectively. The LOD data measured in low osmolar samples were 43.57 mOsm/kg and 65.15 mOsm/kg in Lot A and Lot B, respectively (Figure 1).

3.3 | Linearity

The linearity between the osmolality measured by the UF-5000, referred to as measured, and the manually calculated values, referred

to as assigned, is shown in Table 2. The five concentrations to assess linearity were included in the reference range (300-850 mOsm/kg) of the osmolality. The coefficient of correlation (R) of the linear equation (y = 1.097x + 16.91) was 0.997. The linearity was evaluated by the absolute difference from the most suitable non-linear curve to the linear regression equation. Deviation of linearity in the lowest and highest concentration was −14.79 mOsm/kg and −14.80 mOsm/kg, respectively, which were within the allowable limit of ±15.0 mOsm/kg.

3.4 | Method comparison

The 270 urine specimens were tested using OsmoPro and UF5000 analyzers. The mean (SD) osmolality measured by the OsmoPro and UF5000 was 450.33 (227.51) mOsm/kg and 421.99 (221.53) mOsm/kg, respectively. Passing-Bablok regression analysis showed deviation from the equality line between the OsmoPro and UF5000 assays (Figure 2). There was a moderate correlation between the two assays (coefficient determination R^2 = .667, y = 0.8x + 63.93, where x = OsmoPro vs. y = UF-5000). The mean relative difference in measured urine osmolality between these two assays was −3%. The 95% limit of agreement (LoA) ranged from −70.50% to 55.06%. The mean absolute difference in the measured urine osmolality between these two assays was −28.3 mOsm/kg, and the 95% LoA was from −295.13 mOsm/kg to 238.45 mOsm/kg. The allowable difference between the two analyzers is 28.3%, which is subject to biologic variation as quantified by the west guard database. In this study, 106 among the 270 samples were outside the allowable range. The UF-5000 measurements showed a weak level of agreement (Cohen's kappa = 0.54, 95% CI, 0.45-0.63) with the measurements from the OsmoPro assay. They had an overall agreement of 200/270 (74.1%) among all samples (Table 3).

4 | DISCUSSION

The UF-5000 automated urine sediment analyzer contains a fluorescence flow cytometry detector that can count and differentiate

FIGURE 2 A, Bland-Altman relative difference plot of 270 random urine specimens for measuring osmolality by the UF-5000 and the OsmoPro assay. The continuous line represents the mean difference. The broken lines (blue) represent 95% limit of agreement (LoA) from the mean. The broken lines (gray) represent allowable difference for comparative methods. B, Bland-Altman absolute difference plot of 270 random urine specimens for measuring osmolality by the UF-5000 and the OsmoPro assay. The continuous line represents the mean difference. The broken lines (blue) represent 95% limit of agreement (LoA) from the mean. The broken lines (gray) represent allowable difference for comparative methods
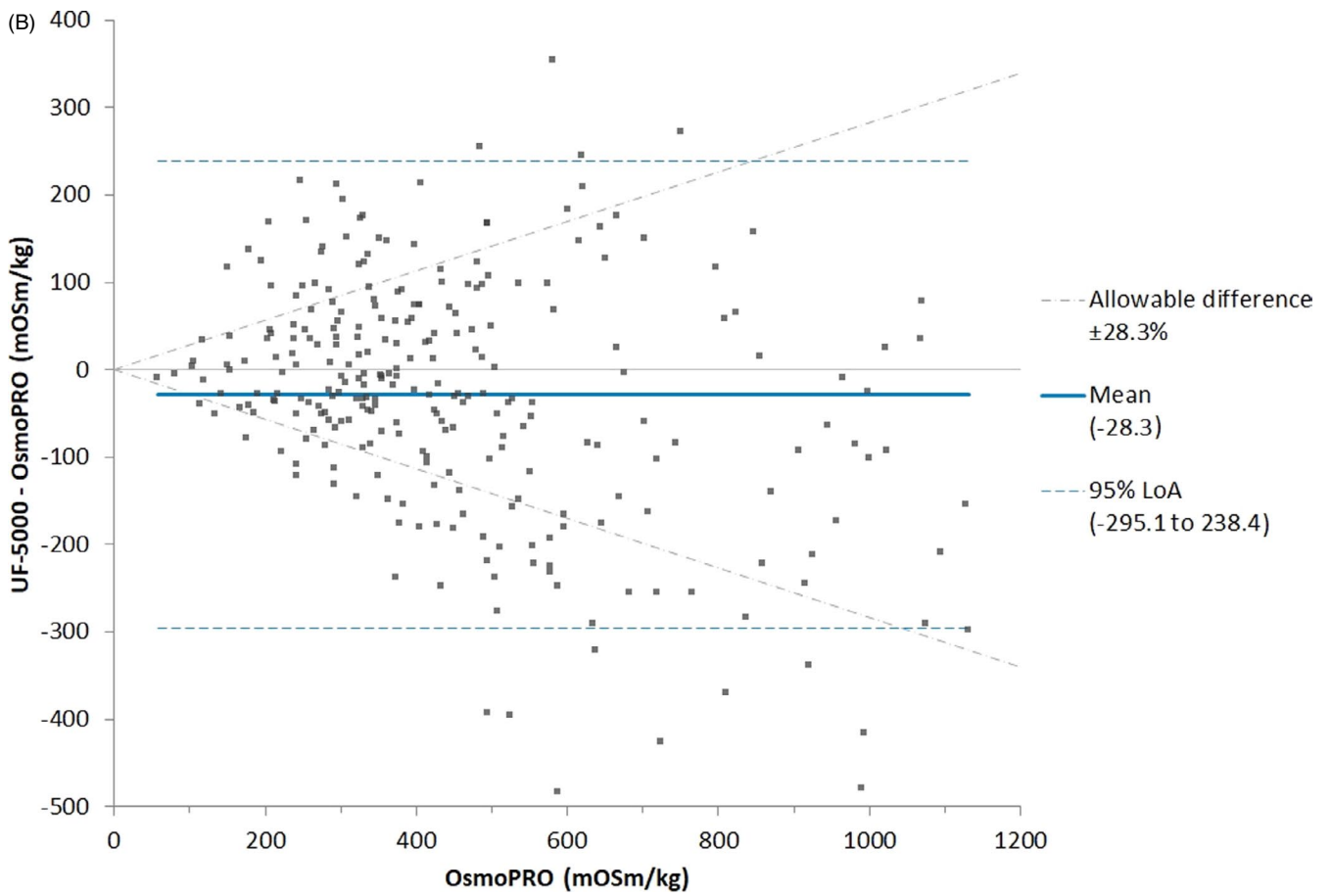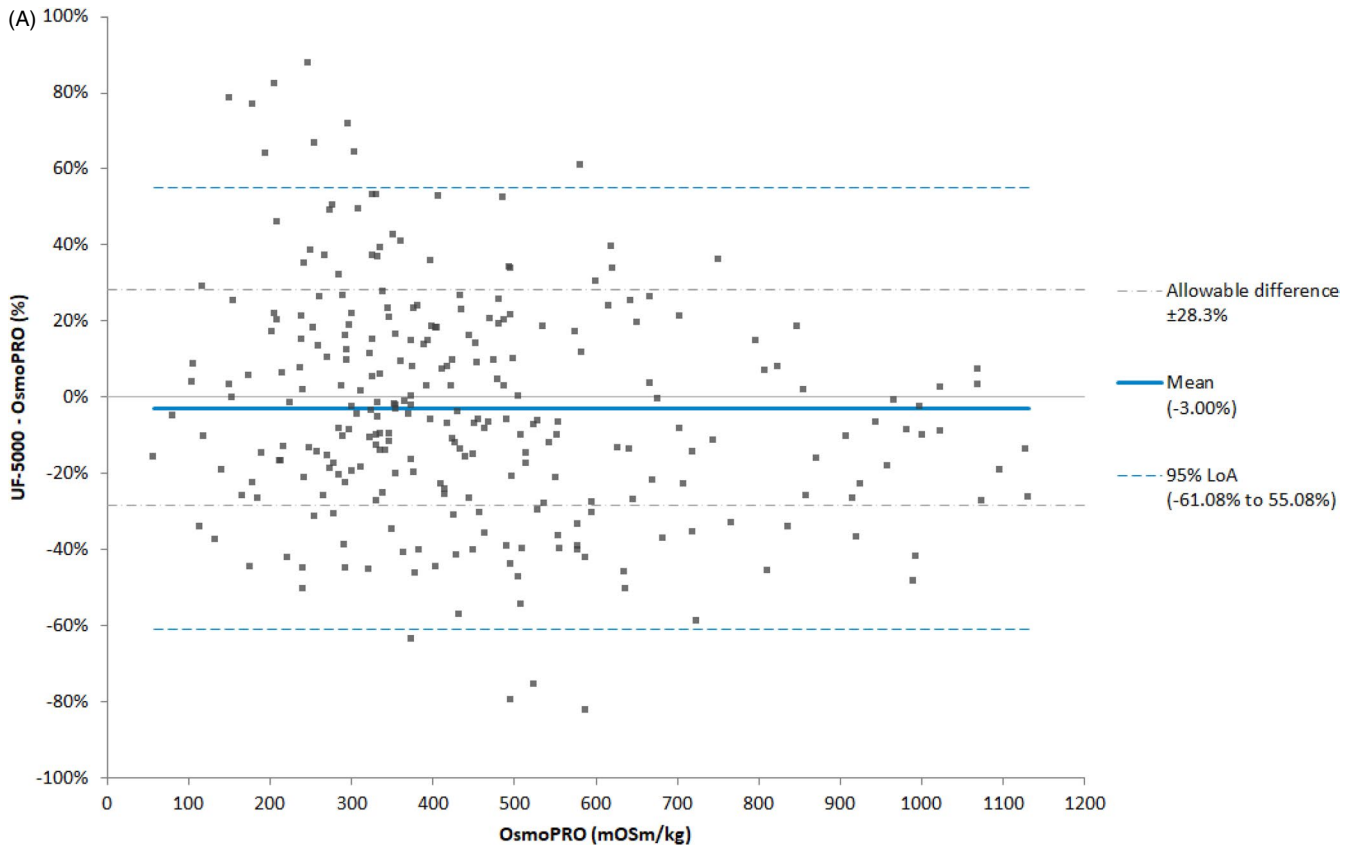
TABLE 3 Comparison of OsmoPro and UF-5000 assays for measurement of urine osmolality

		OsmoPro (N of samples)			
		<300 mOsm/kg	300 ~ 850 mOsm/kg	>850 mOsm/kg	Total
UF-5000 (N of samples)	<300 mOsm/kg	54	32	0	86 (31.9%)
	300 ~ 850 mOsm/kg	19	134	11	164 (60.7%)
	>850 mOsm/kg	0	8	12	20 (7.4%)
	Total N (%)	73 (27.0%)	174 (64.4%)	23 (8.5%)	270

between various cells, crystals, casts, and bacteria found in urine. It also measures urine osmolality using electrical conductivity. We evaluated the precision of the UF-5000 using commercial reagents and actual patient samples. At both reagents and patient samples, the CV values same-day, day-to-day, and within the laboratory were lower than 5%. In one study evaluating an osmolality analyzer using the standardized freezing point method, the precision result was reported to be less than 5%,[10] which is thus supportive of our results.

The UF-5000 showed acceptable linearity over some of the evaluated concentrations. The linearity range within our evaluation encompassed the provided reference range (300-850 mOsm/kg). At all concentrations, the absolute difference between the linear and polynomial fits was less than ± 15 mOsm/kg of allowable non-linearity. These results suggest that the calculation of conductivity could follow a linear pattern within the reference range of osmolality.

The two analyzers were evaluated to determine whether the urine osmolality measurements correlated, in which the absolute mean difference (mOsm/kg) and the relative mean difference (%) between the two analyzers were calculated. A study compared urine osmolality measured by the UF-5000 analyzer to that of OM-6050 (Osmo Station OM-6050; ARKRAY; Menarini Diagnostics) automatic osmometer that uses a freezing point depression method.[2] The correlation between the two analyzers generated an $R^2 = .539$, which also had a moderate correlation, but it was lower than the value ($R^2 = .667$) in our study. This coefficient determination was also less than the 0.975 required by CLSI EP9-A3. The difference between the two analyzers is neither one-sided, nor proportional to the osmolality. Actually in our study, Cohen's kappa value (0.54) for the detection of each interval was not very high. It may result from the measurement methods utilized. During the freezing point depression method, all the materials in solution could affect the osmolality. Within the conductivity method, only the charged materials contribute to the measured osmolality.[5,11] According to Kohlrausch's law, the electrical conductivity is affected by the concentration of all the charged particles in the urine.[12] Therefore, if uncharged particles such as glucose, urea, and contrast agents are present in the urine, it can lead to inaccurate results when measuring the conductivity of the urine.

Our study had several limitations. There is a difference in the detection capability, signified by LOB and LOD, of the two different reagent lots used. The distilled water of Lot A and B is different. Different distilled water may have different levels of resistance. Measurement methods based on conductivity can lead to differences. The presence of RBCs, WBC, or microbes in urine may affect the osmolality readout from the OsmoPro.[2] Therefore, the osmolality values may differ between the two analyzers in the presence of kidney disease, urine contamination, or urinary tract infection. Our study did not evaluate factors that could reduce this correlation. Future studies are required to assess the characteristics of patient groups, such as diagnosis and the development of urethritis and hematuria. In addition, the urine samples containing the non-charged substances may produce an osmolality readout lower than the actual osmolality, so the ratio of each substance contained in the urine is an important factor. This study did not measure the relative or absolute quantities of these non-charged materials in the urine samples. This study evaluated the analytical performance of the urine osmolality measured on the routine analyzer UF-5000. The conductivity-based measurement of osmolality has reliable precision and linearity within a specific range that includes the reference range. Considering the good accessibility of routine analyzer, UF-5000 can be used to determine whether the urine osmolality is within the reference or should be measured by freezing depression method.

AUTHOR CONTRIBUTIONS
DW Yoo contributed to writing—Original Draft, Formal analysis, Data curation. SY Moon contributed to software for figures and statics, and writing—review and editing. SM Lee contributed to conceptualization, study design, writing—methodology, review and editing. IS Kim contributed to investigation, and writing—review and editing. CL Chang contributed to project administration, and writing—review and editing.

ETHICAL APPROVAL
This study was approved by the Institutional Review Board of our hospital after full committee review (approval no. 05-2018-134).

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