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Refrigerated and Room Temperature Storage Stability of Urine Osmolality Measurements

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ABSTRACT

Urine osmolality, a measure of urine concentration, is used to help evaluate the body's water balance. It is typically monitored to evaluate renal function, polyuria and oliguria. This study was conducted to quantify the effects of storage temperature, duration, and sample treatment on urine osmolality measurements. Twenty different human urine samples, including 10 female and 10 male, were collected without preservatives using the clean catch urine collection procedure. Each of the 20 samples was divided into 2 aliquot types, one for neat testing and one centrifuged at 3000 rpm for 10 minutes for supernatant testing, and stored at room (22°C) or refrigerated (4°C) temperature until time of test. All samples were tested for osmolality in triplicate using the Advanced® 3250 Single-Sample Osmometer; the initial readings were considered the baseline, hour zero readings. All urine samples at each temperature were tested at 4, 24 and 48 hour time intervals. The sample mean was calculated, rendering 80 data points for the hour 4, 24, and 48 time points. Percent bias for room temperature and refrigerated urine samples at delayed time points were calculated. Baseline, hour zero osmolality for supernatant urine samples ranged from 187.3 - 1144.0 mOsm/kg H_2O, and from 187.3 - 1142.3 mOsm/kg H_2O for neat urine samples. For neat and supernatant samples stored at both room and refrigerated temperatures, 1 out of 80 samples tested at hour 4 had a percent bias of > 0.5%; 5 out of 80 samples tested at hour 24 had a percent bias of > 0.5%; and 20 out of 80 samples at hour 48 had a percent bias of > 0.5%. Standard practice indicates that specimens should be tested as soon as possible, or refrigerated within 4 hours and tested within 24 hours of collection to obtain accurate osmolality measurements. This data demonstrates stable urine osmolality results for both supernatant and neat samples at either room or refrigerated temperature from baseline up to 24 hours. No appreciable difference between neat and supernatant samples was observed.

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INTRODUCTION

Principle of osmolality

Osmolality, expressed in units of osmoles of solute per kilogram of solvent (or, more commonly, milliosmoles per kilogram) corresponds to the osmotic pressure of a solution. It relies only on the total number of the solutes in a solution and not on solute size or type. Because osmolality depends on molal concentration and not on mass concentration, it is a useful determination of total solute concentration in biological solutions such as urine, whole blood, serum, and plasma[1].

Clinical Significance of Urine Osmolality

The osmolality of a solution plays a key role in intracellular and extracellular volume and tonicity. Since this homeostasis can be affected by many poisons, medications and diseases, osmolality measurements can be an important diagnostic tool.[2] Osmolality testing is conducted on a urine sample collected first thing in the morning, multiple timed samples, or on a cumulative sample collected over a 24-hour period.

Kidneys that are functioning normally will excrete water in relation to the amount consumed. Healthy kidneys should be able to concentrate urine to an osmolality 4 times that of serum, as well as be able to dilute to an osmolality ¼ that of serum osmolality. Those with failing kidneys may not be able to concentrate urine. Solutes will equilibrate by passive diffusion in the renal tubules and the osmolality will be the same as plasma, approximately 290 mOsm/Kg[3]. Unlike urine osmolality, specific gravity is affected by both the number and size of particles in solution. Large molecules like glucose, proteins and foreign substances such as dyes, methanol, ethanol, isopropyl alcohol, acetone and ethylene glycol can invalidate urinary specific gravity results. Therefore, urine osmolality is a more accurate measurement for evaluating the ability of the kidneys to concentrate or dilute the urine. In addition, clinicians often compare urine and serum osmolality measurements in patients to obtain a more accurate assessment of fluid homeostasis.

Urine osmolality is a useful tool in identifying sodium imbalance, and evaluating renal function, polyuria, and oliguria. Conditions that may lead to increased urine osmolality are dehydration, syndrome of inappropriate ADH secretions (SIADH), adrenal insufficiency, glycosuria, hypernatremia, and a high protein diet. Conditions that may lead to decreased urine osmolality are diabetes insipidus, excess fluid intake, acute renal insufficiency, and glomerulonephritis[3,4].

Random urine osmolality specimens can range from 50 to 1400 mOsm/kg H_2O, with an average range of 500 to 800 mOsm/kg H_2O. Urine osmolality should be at least 3 times the serum osmolality following an overnight fast. After restricted fluid intake, the urine osmolality should be >850 mOsm/kg H_2O[3].

Factors that may affect the accuracy of urine osmolality results include test method, specimen collection, and storage temperature and duration[1,5,6].

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Obtaining Specimens for Osmolality Testing

To reduce significant impact on analytical test results, specimen collection and handling should be consistent[7]. For this reason, standard operating procedures for urine collection were followed.

Urine was collected in sterile specimen collection containers by volunteers who followed the Clean Catch Urine Collection Procedure[8]. Enough urine was collected to test 3 replicates each of refrigerated and room temperature samples, both supernatant and neat.

Although particulate matter does not affect the osmolality results, it is possible that it could cause freeze errors or erratic results from the osmometer. Some urine samples may require centrifugation to remove gross particulate matter from the urine sample[1].

Storage Temperature and Duration of Urine Specimens

It is well established that storage temperature and duration affect the accuracy of urine osmolality results[1,5,6]. Guidelines for storage temperature and duration, however, are conflicting in the literature. Claims for the stability of urine osmolality include: "Urine osmolality has been reported to be stable for up to 24 hours at 4°C, and as long as a week when frozen in a sealed container."[5], "If analysis cannot be carried out soon after collection, the specimen should be refrigerated."[7], "Urine osmolality may be stable at 4°C for up to 24 hours. Frozen samples may be stable for several weeks."[4], and "Urine specimens are stable for 24 hours at 4°C or 30°C. Frozen (urine) specimens are stable for 1 week…if there is a delay in the analysis, refrigerate the capped specimens to avoid a change in the original solute concentration."[1].

The objective of this study was to investigate the influence of storage time and temperature on the stability of human urine osmolality measurements using the Advanced® 3250 Single-Sample Osmometer.

MATERIALS AND METHODS

Twenty different human urine samples from 10 female and 10 male volunteers were split into two aliquots, one neat and one centrifuged to remove particulates. Aliquoted samples were stored in VWR disposable, round bottom, sterile, 17 x 100mm culture tubes with closures. These culture tubes were also used for centrifuging the aliquots. Urine collected in sterile specimen collection containers was used to determine color and turbidity, and tested for leukocytes, nitrates, urobilinogen, protein, pH, blood, specific gravity, ketones, bilirubin, and glucose, using Multistix 10SG urine test strips.

Aliquots were centrifuged at 3000 rpm for 10 minutes using a Hettich EBA 3S Table Top Centrifuge. The supernatant was poured off into a new culture tube, being careful not to disturb the pellet. These 40 samples were immediately tested in replicates of 3 using the Advanced® 3250 Single-Sample Osmometer (Advanced Instruments, Norwood, MA). These readings were considered the baseline, hour zero readings.

Each of the supernatant and neat samples were then split into two additional aliquots, one stored at room temperature (22°C) and the other refrigerated (4°C). Room temperature and refrigerated samples were tested at 4, 24, and 48 hour intervals following hour zero testing to simulate delayed sample processing. Refrigerated samples were warmed to room temperature prior to testing[7]. All samples were tested in triplicate. The arithmetic means for room temperature and refrigerated urine samples tested at hours 4, 24, and 48 were calculated. These values were compared to their corresponding hour zero values to calculate percent bias. Microsoft® Excel 2007 was used for statistical analyses.

"Random urine osmolality specimens can range from 50 to 1400 mOsm/kg H_2O, with an average range of 500 to 800 mOsm/kg H_2O."

RESULTS

Table 1. Neat and Supernatant 22˚C

	Human Serum Sample	Neat 22˚C				Supernatant 22˚C			
		0 Hours Mean Level (mOsm)	4 Hours % Bias	24 Hours % Bias	48 Hours % Bias	0 Hours Mean Level (mOsm)	4 Hours % Bias	24 Hours % Bias	48 Hours % Bias
Female	1	876.7	-0.16	0.07	0.38	873.3	0.27	0.42	0.69
	2	991	0.23	-0.07	-0.10	991	0.23	-0.10	0.17
	3	810.3	-0.12	-0.32	1.53	809.3	-0.20	-0.32	0.12
	4	1011	-0.07	0.23	-0.07	1012.3	-0.13	0.10	0.20
	5	259.3	-0.13	-0.24	0.37	258.7	-0.15	-0.27	-0.15
	6	248.3	-0.40	-0.52	0.68	248.3	-0.12	-0.52	-0.40
	7	854	-0.04	0.08	0.35	853.7	-0.12	0.39	0.59
	8	719.3	-0.28	0.00	1.63	718.7	-0.33	0.22	1.15
	9	1104.7	-0.18	-0.13	0.24	1103.7	-0.04	0.21	0.51
	10	1142.3	0.21	0.09	0.06	1144	0.15	0.03	-0.06
Male	1	963	0.10	0.00	-0.31	961.7	0.06	0.03	0.00
	2	850	-0.04	-0.08	-0.35	849.7	0.00	-0.24	-0.20
	3	1006	0.00	0.03	0.53	1007	-0.27	0.10	0.30
	4	754.3	0.19	-0.21	-0.17	754.7	0.04	-0.36	-0.23
	5	465.7	-0.21	-0.43	-0.30	465.7	-0.09	-0.15	-0.37
	6	919.3	-0.11	-0.11	0.00	917	0.22	0.00	0.08
	7	828	0.08	0.21	0.33	829	0.04	-0.08	-0.08
	8	911.3	-0.18	-0.03	0.11	911.0	0.00	0.11	0.30
	9	208.7	0.29	-0.34	-0.81	208.3	0.00	-0.29	-1.10
	10	187.3	0.00	-0.32	-0.53	187.3	-0.32	-0.85	-1.07

Table 1. % bias calculations for neat and supernatant samples stored at 22°C

RESULTS (cont.)

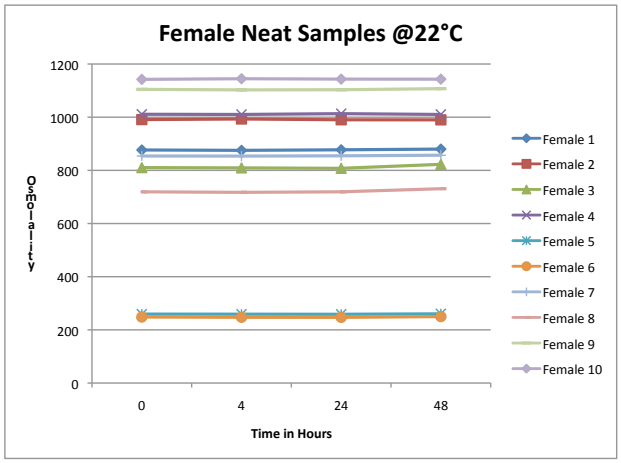

Chart 1. mOsm values over 48 hours for female neat samples stored at 22°C

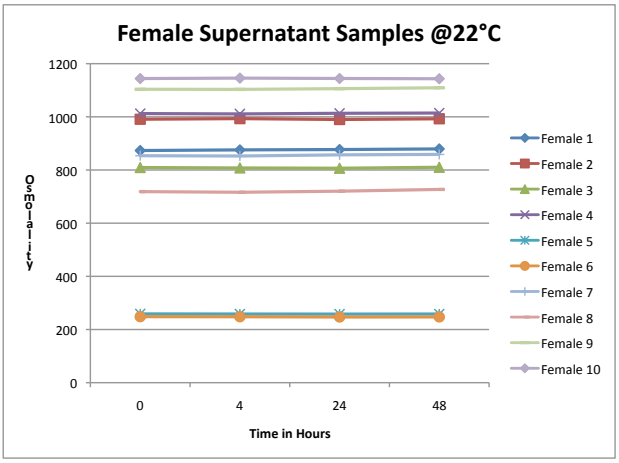

Chart 2. mOsm values over 48 hours for female supernatant samples stored at 22°C

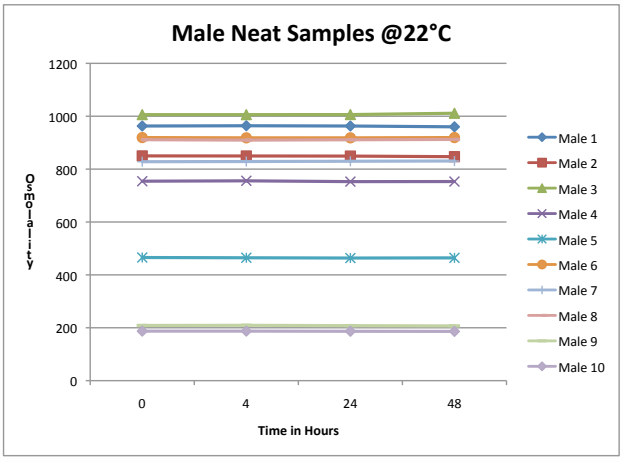

Chart 3. mOsm values over 48 hours for male neat samples stored at 22°C

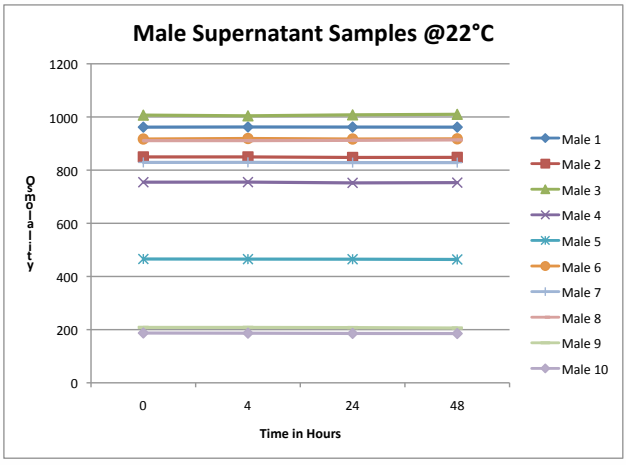

Chart 4. mOsm values over 48 hours for male supernatant samples stored at 22°C

RESULTS (cont.)

Table 2. Neat and Supernatant 4˚C

	Human Serum Sample	Neat 4˚C				Supernatant 4˚C			
		0 Hours Mean Level (mOsm)	4 Hours % Bias	24 Hours % Bias	48 Hours % Bias	0 Hours Mean Level (mOsm)	4 Hours % Bias	24 Hours % Bias	48 Hours % Bias
Female	1	876.7	-0.19	-0.11	-0.16	873.3	0.39	0.31	0.23
	2	991	0.17	-0.07	-0.10	991	0.13	-0.37	-0.17
	3	810.3	-0.04	-0.25	-0.20	809.3	-0.04	-0.12	-0.12
	4	1011	-0.10	0.17	0.37	1012.3	-0.20	-0.13	-0.06
	5	259.3	-0.63	-0.40	-0.40	258.7	-0.27	-0.39	0.23
	6	248.3	0.28	-0.40	-0.52	248.3	-0.40	-0.40	-0.81
	7	854	0.04	0.08	0.12	853.7	0.00	0.07	0.54
	8	719.3	-0.46	-0.32	-0.22	718.7	-0.19	-0.24	-0.10
	9	1104.7	0.18	0.18	0.48	1103.7	0.18	0.24	0.48
	10	1142.3	0.21	0.21	-0.88	1144	0.15	0.06	-0.15
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	3	1006	-0.03	0.00	0.27	1007	-0.13	-0.10	0.23
	4	754.3	0.00	-0.30	-0.17	754.7	0.00	-0.32	-0.32
	5	465.7	-0.15	-0.30	-0.52	465.7	-0.09	-0.43	-0.15
	6	919.3	0.00	-0.11	-0.07	917	0.00	0.08	0.00
	7	828	0.33	0.24	0.00	829	0.16	-0.04	-0.12
	8	911.3	-0.03	0.08	0.26	911.0	0.03	0.00	0.08
	9	208.7	0.14	-0.48	0.96	208.3	0.00	-0.14	-0.14
	10	187.3	0.00	-0.69	-0.69	187.3	-0.16	-0.69	-0.53

Table 2. % bias calculations for neat and supernatant samples stored at 4°C

RESULTS (cont.)

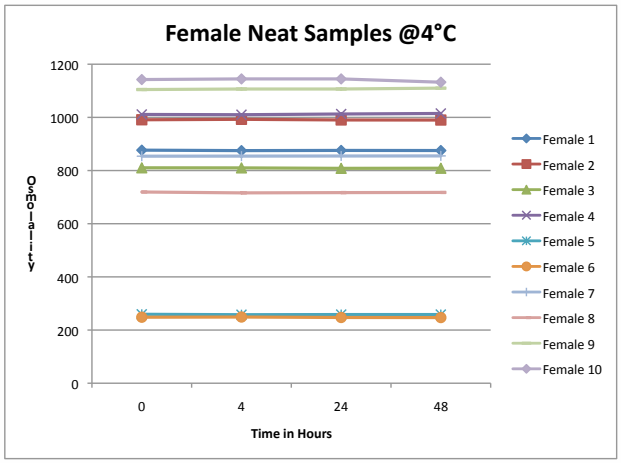

Chart 5. mOsm values over 48 hours for female neat samples stored at 4°C

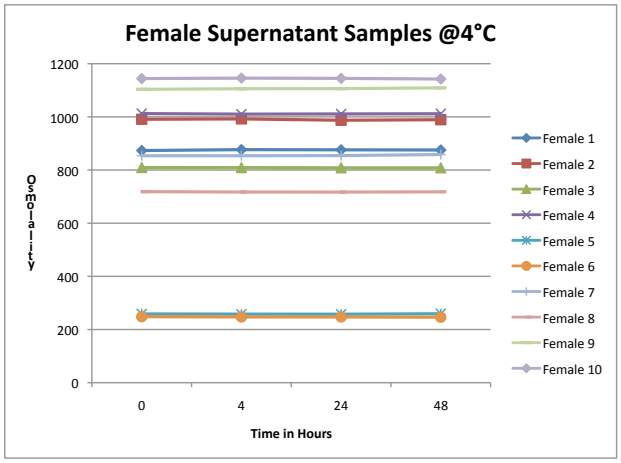

Chart 6. mOsm values over 48 hours for female supernatant samples stored at 4°C

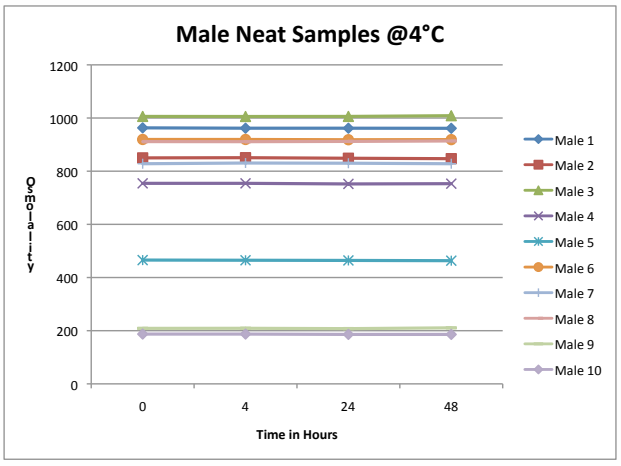

Chart 7. mOsm values over 48 hours for male neat samples stored at 4°C

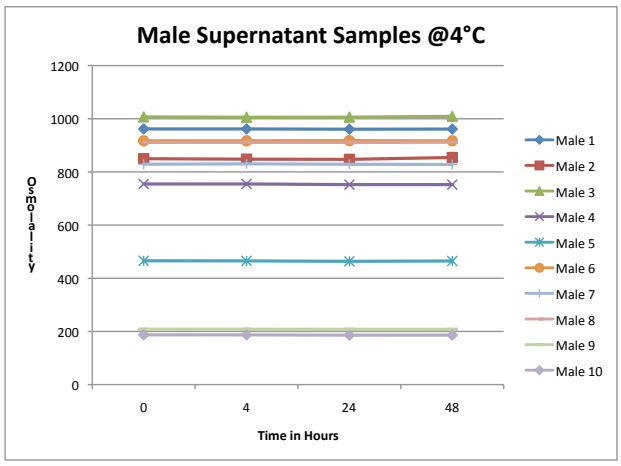

Chart 8. mOsm values over 48 hours for male supernatant samples stored at 4°C

CONCLUSION

Urine specimens should be tested as soon as possible after collection in order to obtain accurate osmolality results in clinical laboratories. Standard practice indicates that specimens should be tested as soon as possible, or refrigerated within 4 hours and tested within 24 hours of collection to obtain accurate osmolality measurements. When immediate processing of urine specimens is not possible, this study suggests that both neat and supernatant human urine samples, either refrigerated or stored at room temperature up to 24 hours, can be accurately tested for osmolality without significant bias. No appreciable difference between neat and supernatant samples was observed.

"When immediate processing of urine specimens is not possible, this study suggests that both neat and supernatant human urine samples, either refrigerated or stored at room temperature up to 24 hours, can be accurately tested for osmolality without significant bias."

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