SCIENTIFIC POSTER

Refrigerated and Room Temperature Storage Stability of Serum Osmolality Measurements

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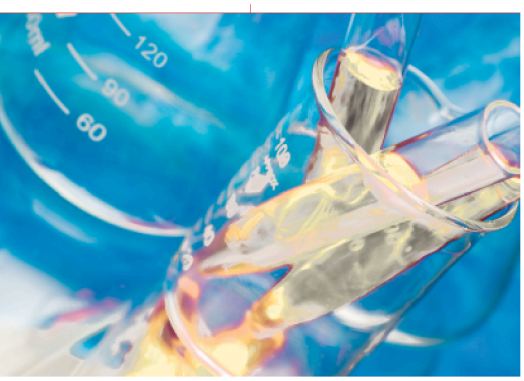

"Serum specimens should be tested as soon as possible after collection in order to obtain accurate osmolality results."

ABSTRACT

The accurate determination of serum osmolality in the clinical laboratory is of critical importance to the differential diagnosis of disorders involving water balance[1]. The temperature at which serum samples are stored prior to processing and the duration of storage affects the stability of osmolality measurements[1,2,3]. This study was conducted to quantify the effects of storage temperature and duration on serum osmolality. Ten different frozen human serum samples were thawed and tested in replicates of 10 for osmolality on the Advanced® 3250 Single-Sample Osmometer. These readings were considered the baseline, hour zero readings. The 10 samples were then separated into two aliquots and stored at room temperature (22°C) or refrigerated (4°C) until time of test. Serum samples at each temperature were tested at hours 4, 24 and 48. Percent bias and standard deviation for room temperature and refrigerated serum samples at delayed time points were calculated. Baseline, hour zero osmolality for human serum samples 1-10 ranged from 265.1 to 294.6 mOsm/kg H_2O. Percent bias for refrigerated and room temperature serum samples 1-10 tested at hour 4 were < 0.5%, meeting the linearity specification for the instrument. The majority of refrigerated and room temperature serum samples at hour 24 and 48 also had a percent bias < 0.5%. Standard deviations for refrigerated and room temperature serum samples 1-10 tested at hour 4, 24, and 48 were < 2 mOsm, meeting the repeatability specification for the instrument. Ideally, specimens should be tested as soon as possible after collection to obtain accurate osmolality measurements. This data demonstrates stable results at either room or refrigerated temperature from baseline up to 48 hours. If immediate processing is not possible, however, it is recommended that specimens be tested within 24 hours of collection. Additional testing with freshly collected serum will allow for a more definitive claim on samples tested after 24 hours.

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 whole blood, serum, plasma, and urine.

Clinical significance of serum osmolality

Normal human serum has an osmolality in the range 278-305 mOsm[1]. The assessment of serum osmolality in clinical laboratories is critical to the differential diagnosis of disorders involving water and solute balance in the blood. Most commonly, osmolality measurements are used to help determine if water loss is occurring in excess of solute loss, resulting in an increased serum osmolality[4]. Elevated serum osmolality can be seen in cases of renal failure (hyperuremia)[4], dehydration[5], and diabetes (hyperglycemia)[5]. Mannitol therapy[6] and toxin ingestion[7] may also increase serum osmolality. Less frequently, osmolality measurements are used to determine if a patient is in a hypo-osmolar state. Serum osmolality often decreases in cases of overhydration[4] and inappropriate secretion of antidiuretic hormone[5].
It is important to obtain true serum osmolality measurements in order to make accurate diagnoses. Several factors that affect the accuracy of serum osmolality results include test method[8], specimen collection[9], and storage temperature and duration[1,2,3].

Measuring osmolality in clinical laboratories

Osmolality is determined using an osmometer. Commercially available osmometers include freezing point, vapor pressure, and membrane osmometers. Freezing point, vapor pressure, and osmotic pressure are all interrelated and mathematically interconvertible, which makes it possible to determine osmotic pressure by measuring freezing point or vapor pressure[5].

Freezing point osmometers and vapor pressure osmometers employ freezing point depression and vapor pressure depression, respectively. The addition of 1 mole of solute to 1 kilogram of water depresses the freezing point of a solution by 1.86°C and depresses the vapor pressure by 0.3 mmHg (40 Pa). Membrane osmometers measure the osmotic pressure of a solution separated by a semi-permeable membrane. The addition of 1 mole of solute to 1 kilogram of water increases the osmotic pressure of a solution by 22.4 atmospheres. Freezing point, vapor pressure, and membrane osmometers each have unique advantages and disadvantages[8]. Freezing point depression is the method preferred by clinical laboratories for measuring osmolality because it is rapid and more precise than both vapor pressure and membrane osmometry[10,11].

Comparison of commercially available osmometers

Osmometer Type:	Advantages:	Disadvantages:	Comments:
Freezing Point Osmometry (FPO)	• Performs rapid and inexpensive measurements • Simple and reliable performance • Industry-preferred FP method • Small sample size (nL to µL range) • Ideal for dilute biological and aqueous solutions • Independent of changes in ambient temperature	• Samples must be of low viscosity • Not ideally suited for high molality or colloidal solutions	FPO provides rapid and inexpensive results with the industry-preferred freezing point method. Requires small sample size and ideally suited for most biological and aqueous applications.
Vapor Pressure Osmometry (VPO)	• Performs rapid and inexpensive measurements • Small sample size (nL to µL range) • Ideal for dilute biological and aqueous solutions	• Less accurate than FPO • Cannot be used for volatile solutes like alcohols or other organic solvents • Not ideally suited for high molality or colloidal solutions	VPO provides fast and inexpensive measurements requiring a small sample size, although not as accurate as FPO.
Membrane Osmometry (MO)	• Provides potentially unlimited direct measurement of osmotic pressure and solution osmolality • Good for colloidal solutions • No limitation on sample concentration • Can determine molecular weight of macromolecules	• Time consuming and difficult to operate • Requires large sample volume • Not applicable for small molecules and aggressive solvents due to membrane porosity and compatibility • Irreproducible results due to clogging of membrane pores	MO provides a direct measurement of osmolality and is suitable for high molality and colloidal samples. Long analysis time, requires large sample volumes. Not applicable for small molecule applications.

INTRODUCTION (cont.)

The Advanced 3250 Single-Sample Osmometer uses the freezing point method for an accurate and reliable determination of the osmolality of a 0.2 to 0.25 mL sample in approximately 2 minutes. The instrument specifications include linearity that is less than ±0.5% from a straight line and repeatability with a standard deviation of < 2 mOsm/kg H_2O between 0 and 400 and < 0.5% of value in mOsm/kg H_2O when operated between 20°C to 25°C and 40% to 60% relative humidity[8].

Obtaining specimens for osmolality testing

Small differences in specimen collection and handling can have a significant impact on analytical test results[9]. For this reason, standard operating procedures for serum collection should be followed. Enough blood to run a series of 0.25 mL tests should be collected by venipuncture with minimal stasis[5]. Blood drawn without the use of any anticoagulant (red top tube) is preferred[4]. Needles, syringes, and tubes used to collect blood should be clean and dry[3]. Blood should be allowed to clot at room temperature for 30-60 minutes after collection. Specimens that are not given at least 30 minutes to clot are likely to retain cellular contaminants which may affect osmolality measurements. After 60 minutes, cells in the clot are likely to lyse, compromising sample integrity[9]. Serum should be separated from blood cells and clotting factors by centrifugation. Serum may require additional centrifugation to remove particulate matter from the serum sample which can act as a seeding agent and cause premature crystallization[3].

Storage temperature and duration of serum specimens

It is well established that storage temperature and duration affect the accuracy of serum osmolality results[1,2,3]. Guidelines for storage temperature and duration, however, are conflicting in the literature. Claims for the stability of serum osmolality include: *"The osmolality of serum does not alter over a period of 1-2 hours either at room temperature (20°C) or at 37°, but if serum is stored overnight at 4°, the osmolality falls by 1-2 mOsm"*[2]; *"Specimens remain unchanged for 3 hours at room temperature and for 10 hours at 4°C"*[3]; and *"Serum specimens are stable for 3 days at 4°C and 30°C"*[1]. The objective of this study was to investigate the influence of storage time and temperature on the stability of human serum osmolality measurements using the Advanced 3250 Single-Sample Osmometer.

MATERIALS AND METHODS

Ten different frozen human serum samples from SeraCare Life Sciences, Inc. (Milford, MA) were thawed and tested in replicates of 10 using the Advanced 3250 Single-Sample Osmometer (Norwood, MA). These readings were considered the baseline, hour zero readings to simulate sample processing at time of collection. Each sample was then split into two aliquots, one stored at room temperature (22°C) and the other refrigerated (4°C). Aliquoted samples were stored in VWR disposable, round bottom, sterile, 17x100mm culture tubes with closures. Room temperature and refrigerated samples were tested at hours 4, 24, and 48 to simulate delayed sample processing. Samples were tested in replicates of 10, with the exception of room temperature samples 6-10 at 4 hours, refrigerated samples 1-10 at 4 hours, and refrigerated samples 4 and 5 at 24 hours, which were tested in replicates of 5. The arithmetic mean and standard deviation for room temperature and refrigerated serum samples tested at hours 4, 24, and 48 were calculated. Arithmetic means were used to determine percent bias for room temperature and refrigerated samples tested at delayed time points relative to hour zero. We evaluated whether changes in osmolality due to delayed sample processing were significant by comparing calculated percent bias and standard deviation to the linearity and repeatability specifications for the Advanced 3250 Single-Sample Osmometer. Microsoft® Excel 2009 was used for statistical analyses.

RESULTS

Baseline, hour zero osmolality for human serum samples 1-10 ranged from 265.1 to 294.6 mOsm. Serum samples 2 and 9, with baseline osmolalities of 265.1 and 275.1 mOsm, respectively, fell outside of the normal range.

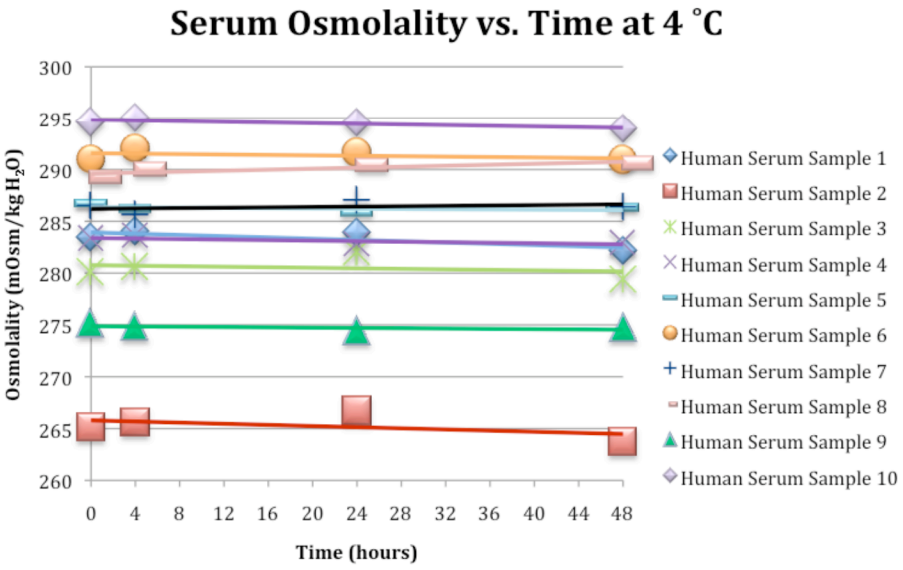

Figure 1. Average osmolality of refrigerated (4°C) human serum samples at hours 0, 4, 24, and 48.

Figure 1 exhibits the trend in osmolality for refrigerated (4°C) serum samples 1-10 from hour zero up to 48 hours. Eight out of 10 refrigerated samples showed a slightly decreasing trend in osmolality from 0 hours to 48 hours.

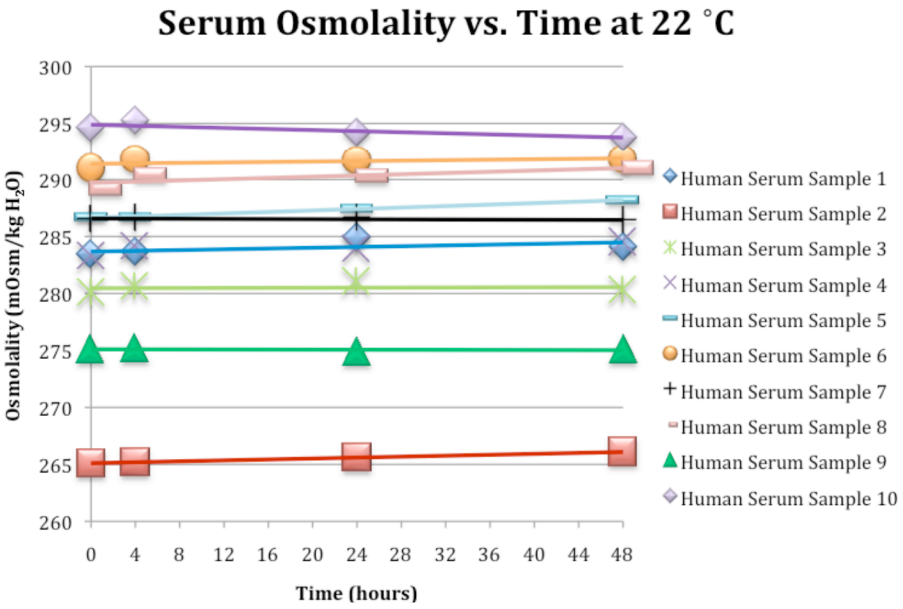

Figure 2. Average osmolality of room temperature (22°C) human serum samples at hours 0, 4, 24, and 48.

Figure 2 exhibits the trend in osmolality for room temperature (22°C) serum samples 1-10 from hour zero up to 48 hours. No common increasing, decreasing, or stabilizing trend in the osmolality of room temperature samples 1-10 was observed.

RESULTS (cont.)

Table 1 summarizes the percent bias for refrigerated and room temperature human serum samples tested at delayed time points. Percent bias for refrigerated and room temperature serum samples 1-10 tested at hour 4 were < 0.5%, meeting the linearity specification for the Advanced 3250 Single-Sample Osmometer. The majority of refrigerated and room temperature serum samples at hour 24 and 48 also had a percent bias < 0.5%.

Human Serum Sample	0 Hours Mean Level (mOsm)	Refrigerated (4 ˚C)			Room Temperature (22 ˚C)		
		4 Hours % Bias	24 Hours % Bias	48 Hours % Bias	4 Hours % Bias	24 Hours % Bias	48 Hours % Bias
1	283.4	0.21	0.18	-0.46	0.07	0.53	0.25
2	265.1	0.19	0.60	-0.53	0.04	0.19	0.38
3	280.1	0.18	0.68	-0.25	0.18	0.32	0.07
4	283.3	0.11	-0.18	-0.14	0.28	0.21	0.42
5	286.7	-0.17	-0.52	-0.14	0.00	0.24	0.52
6	291.0	0.34	0.21	-0.03	0.27	0.24	0.27
7	286.5	-0.31	0.17	-0.03	0.03	0.03	-0.03
8	289.2	0.28	0.41	0.48	0.41	0.38	0.62
9	275.1	-0.11	-0.25	-0.15	0.04	-0.07	0.00
10	294.6	0.14	-0.03	-0.20	0.20	-0.14	-0.27

Table 1. Percent bias for refrigerated and room temperature human serum samples tested at delayed time points, hour 4, 24, and 48.

Table 2 summarizes the standard deviation for refrigerated and room temperature human serum samples tested at delayed time points. Standard deviations for refrigerated and room temperature serum samples 1-10 tested at hour 4, 24, and 48 were < 2 mOsm, meeting the repeatability specification for the Advanced 3250 Single-Sample Osmometer.

Human Serum Sample	0 Hours St. Dev (mOsm)	Refrigerated (4 ˚C)			Room Temperature (22 ˚C)		
		4 Hours St. Dev	24 Hours St. Dev	48 Hours St. Dev	4 Hours St. Dev	24 Hours St. Dev	48 Hours St. Dev
1	0.8	0.7	0.7	1.0	1.0	1.1	1.4
2	0.6	1.1	0.8	1.1	0.6	0.7	0.9
3	1.2	0.5	0.7	1.1	0.5	0.7	0.9
4	0.7	0.5	0.4	0.9	0.9	0.6	1.0
5	0.7	0.4	0.4	1.1	0.8	0.7	1.4
6	0.5	0.0	0.5	0.3	0.4	0.5	0.9
7	0.5	1.5	0.7	0.5	0.5	0.5	0.5
8	1.3	0.0	0.8	0.5	0.5	0.5	0.5
9	0.6	0.4	0.5	0.5	0.4	0.3	0.3
10	1.0	0.0	0.5	0.5	0.4	0.4	0.4

Table 2. Standard deviations for refrigerated and room temperature human serum samples tested at delayed time points, hour 4, 24, and 48.

CONCLUSION

Serum specimens should be tested as soon as possible after collection in order to obtain accurate osmolality results in clinical laboratories. When immediate processing of serum specimens is not possible, this study suggests that human serum samples refrigerated or stored at room temperature for up to 48 hours can be accurately and precisely tested for osmolality without significant bias. It is recommended, however, that users test serum specimens within 24 hours of collection. Additional testing with freshly collected serum will allow for a more definitive claim on samples tested after 24 hours.

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