Osmolality – Serum, Plasma, Urine
Advanced Micro-Osmometer Model 3320

Technical Procedure 3338

Principle

Intended Use

Advanced® Micro-Osmometer Model 3320 uses the technique of freezing-point depression to measure osmolality in serum, plasma and urine. Osmolality is the total solute concentration of an aqueous solution. Osmometers measure the number of solute particles irrespective of molecular weight or ionic charge.

Clinical Significance

Osmolality results assist in establishing the proper diagnoses and treatments for patients with disorders involving water and electrolyte imbalances.

Serum Osmolality

Hyperosmolality not due to hypernatremia indicates an excess of another solute. In most cases, this condition results from increases in glucose and urea. Increased ketone bodies in a diabetic and starvation acidosis may increase osmolality by 5-10 mOsm/kg or more. Calcium, magnesium and potassium changes do not affect osmolality significantly. Conditions causing these results may be liver failure, shock, severe infections, toxemia, carcinoma, poisoning, uremia, hyperglycemia, diabetic acidosis. Serum osmolality may be used to screen for alcohol poisoning by means of the osmolal gap. A significant disparity (>10 mOsm/kg) between measured and calculated osmolality may corroborate the diagnosis of toxic alcohol ingestion.

Hypernatremia is always accompanied by hyperosmolality. Dehydration that results in hypernatremia may be caused by diarrhea in infants, prolonged diarrhea in adults, and peritonitis or intestinal obstruction in which fluid is sequestered outside the vascular system. Excess exogenous salt is another cause and could result from over-administration of saline solutions, especially in newborns. Decreased fluid intake, especially in the elderly, also results in this condition.

Hypo-osmolality is associated with some degree of hyponatremia. This finding in conjunction with a normal serum sodium and serum osmolality indicates there is more water than normal relative to sodium. This may occur in several ways:

a. Acute diarrhea or vomiting with subsequent rapid water replacement.
b. Post-operatively following anesthesia.
c. Acute alcoholic intoxication-alcohol induced ADH depression produces diuresis and electrolyte excretion; resulting thirst leads to over hydration with electrolyte replacement.

Chronic hyponatremia may result from congestive heart failure, inappropriate ADH secretion, and head injury. This is dilutional hyponatremia.

Urine Osmolality

Osmolality seems preferable to specific gravity as a measure of kidney function. Osmolality is only minimally affected by the presence of protein, glucose, radio-opaque substances, or diet in contrast to specific gravity. Osmolality may be an important tool in following the course of progressive kidney failure by means of the serum/ urine osmolality ratio. Normally this ratio is less than one but in kidney disease it remains close to 1.0.

Acute renal failure may be differentiated from severe dehydration. In the former, urine osmolality approaches that of serum. Thus, if urine osmolality is greater than 500 mOsm/kg, probable cause is not renal failure.

Polyuria may result from several conditions. In diabetes insipidus, urine osmolality is low with a high serum/urine osmolality ratio. Patients with diabetes insipidus respond only slightly to deprivation whereas the patient with polydypsia will show urine osmolalities of 800 to 1000 mOsm/kg.

High solute syndrome and chronic edema may occur in congestive heart failure yielding low urine osmolality (200-600 mOsm/kg).

Event	Serum Osmolality	Urine Osmolality
Post surgery	Decreased	Increased
Hepatic cirrhosis	Decreased	Increased
Congestive heart failure	Decreased	Increased
Diabetes insipidus	Increased	Decreased
Diabetes mellitus	Increased	Increased
High protein diet	Increased	Increased
Excess water loss	Increased	Decreased
Increased solute intake	Increased	Increased
Chronic renal disease	Increased	Decreased
Dehydration	Increased	Increased
Excess water intake	Decreased	Decreased
Diuretics	Increased	Decreased
Adrenal insufficiency	Increased	Increased

Urine/Serum Osmolality Ratio

Normally, with average fluid intake, this ratio is between 1 and 3. It rises above 3 in concentration tests or during fluid restriction to as high as 4.7.

Methodology

The Advanced Mico-Osmometer method is based on the principles of Freezing-Point Osmometry

When a solute is dissolved in a pure solvent, the following changes in the solution's properties occur:

- the freezing point is depressed,
- boiling point is raised,
- osmotic pressure is increased, and
- vapor pressure is lowered.

These are the “colligative” or concentrative properties of the solution which, within reasonable limits, change in direct proportion to the solute concentration; more specifically, the number of particles in solution.

Of the colligative properties, measurement of the freezing point allows the concentration of an aqueous solution to be easily determined with great precision.

The freezing point of pure H2O is precisely +0.010°C. One mole of a non-dissociating solute such as glucose (where the solute does not dissociate into ionic species, but remains intact), when dissolved in 1 kilogram (kg) of water will depress the freezing point by 1.858°C. This change is known as the freezing point depression constant for water. The freezing point depression also depends upon the degree of dissociation of the solute. If the solute is ionic, the freezing point is depressed by 1.858°C for each ionic species. For example, if one mole of sodium chloride were to completely dissociate into two ionic species (Na+ and Cl-) in 1 kg of water, the freezing point would be depressed by 3.716°C. However, dissociation is

never complete. Interference between solute molecules reduces dissociation by a factor called the osmotic coefficient.

In a simple solution such as glucose or sodium chloride in water, the freezing point can be measured and the unit concentration easily determined from an equation or a reference table. However, the equation is unique for each solute. In a more complex solution, all ionized and non-dissociated species contribute to the freezing-point depression and the concentration of each solute cannot be easily determined.

Each of the colligative properties has a similar problem, and though each of the colligative properties changes in direct proportion to the solute concentration, each requires a different mode & unit of measurement. Osmolality is a common unit of concentration measurement that can be used to relate all the colligative properties to each other, and to other concentration units. Because of its universality, most osmometry applications regularly use osmolality, expressed as "mOsm/kg H2O", as the common unit of concentration rather than applying further conversion factors.

Instrumentation

Advanced Osmometers are devices for the determination of the concentration of solutions by means of freezing- point measurement. Advanced Osmometers utilize high-precision thermisters to sense the sample temperature, to control the degree of supercooling and freeze induction, and to measure the freezing point of the sample. They can routinely determine differences of ±1 mOsm/kg H2O.

Freezing-Point Thermodynamics

The quickest and most precise way to measure the freezing point of a solution is to supercool it several degrees below its freezing point. It is unstable in this state, and a mechanical agitation induces crystallization. The heat of fusion suddenly liberated causes the sample temperature to rise toward a plateau temperature, where a liquid/solid equilibrium occurs. The equilibrium temperature is, by definition, the freezing point of the solution. Managing the plateau temperature for precise measurement is the basis for several patents issued to Augustus Fiske.

The time over which liquid/solid equilibrium develops and is maintained, is a function of the speed with which the heat-of-fusion is liberated vs. the speed it is transferred away, or absorbed, by the surrounding environment. This ratio can be slowed and the equilibrium time stretched, to give a distinct plateau height measurable to 0.001°C.

Sensitive thermistor probes monitor the sample temperature and control the thermoelectric cooling element. Micro- processor control and automated operation minimize imprecision due to operator technique.

Fast Cool

The sample has been presented to the thermistor probe and the operating cradle has been pushed down. The sample begins cooling rapidly. The digital display reads 0.

Slow Cool

When the temperature of the sample reaches 0OC, the display begins counting up, and the cooling rate of the sample slows.

Freeze

When the sample has been sufficiently supercooled, a mechanical pulse induces the sample to freeze.

Heat of Fusion

The display counts down very quickly as the heat of fusion escapes the sample and the temperature rises to the actual freezing point of the sample. The display count rate slows as the sample approaches its freezing point.

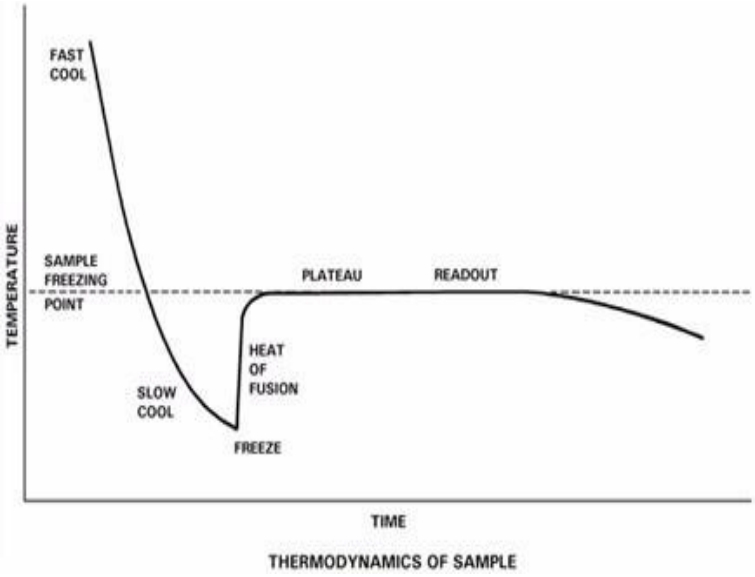
Plateau

The display reading approaches a constant level as the sample reaches thermal equilibrium.

Readout

As soon as the plateau is constant, the sample osmolality is locked in and the display reads Osmolality XXX mOsm. The instrument is now ready for the next test.

The following Standard Freezing Curve illustrates the temperature of a sample as it progresses through the freezing cycle and shows the action of the instrument at each stage of the cycle.



Definitions

Solution: A homogeneous mixture of solute and solvent in which the solvent is usually the major component, and the solute is the minor component.

Concentration: The ratio of solute to a given amount of solvent (molal), or ratio of solute to solution (molar). The amount of solute is usually expressed in terms of moles, i.e., gram molecular weights.
One mole= 6.028 x 10^{23} molecules (Avogadro's number). One mole of glucose (180.2 g) and one mole of sodium chloride (58.4 g) each contain Avogadro's number of molecules.

Common units of concentration include:

Molality: Moles of solute per kilogram of pure solvent.

Osmolality: Osmols of solute particles per kilogram of pure solvent. As noted above, most ionic solutes do not completely dissociate. Osmolality is a unit of concentration that takes into account the dissociative effect. Osmolality is usually expressed in mOsm/kg H2O. One milliosmol (mOsm) is 10^{-3} osmols.

$$Osmolality = \emptyset nC = \frac{osmol}{kg\ H_2O}$$

where:

Ø = osmotic coefficient, which accounts for the degree of molecular dissociation.
n = number of particles into which a molecule can dissociate.
C = molal concentration of the solution.

Molarity: Moles of solute per liter of solution.

Osmolarity: Osmols of solute particles per liter of solution.

Although molarity and osmolarity may be common units of measurement in other branches of chemistry, they are not used in osmometry because the ratio of solute to solution is not linear. Molality and osmolality are linear, independent of the effect of temperature and volume displaced by solute. A calculated conversion between units of molality and molarity is complex and generally unnecessary when the terms are properly understood.

Freezing-Point Depression: When a solute is added to a solvent, the freezing point of the solvent is lowered. In aqueous solutions, one mOsm of solute per kilogram of water depresses the freezing point by 1.858 millidegrees Celsius (m°C).

Supercooling: The tendency of a substance to remain in the liquid state when cooled below its freezing point. Crystallization Temperature: Aqueous solutions can be induced to freeze (i.e., crystallize) most reliably when super-cooled. When supercooled, agitating the solution (freeze pulse) induces crystal formation. The crystallization temperature is the temperature at which crystallization is induced. During crystallization, the heat of fusion raises the temperature of the sample to an ice/water freezing-point plateau.

Heat of Fusion: The heat released when the mobile molecules of a liquid are frozen into rigid ice crystals.

Freezing-Point Plateau: The constant temperature maintained during the time that ice and liquid exist in isothermal equilibrium after crystallization occurs.

Specimen

Acceptable Sample Containers

13 x 75 PST, SST and Red Top BD tubes
PST, SST and Red Top BD microtainers

Spot urines should be aliquoted into a 13x75 Clear Cap BD tube

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum, plasma, or properly collected urine are the preferred specimens.
Whole blood is not recommended for use as a sample.

Unacceptable Specimens

Urine samples aspirated by the IRIS iQ are not acceptable for urine chemistry testing.

Urine collected in a BD UA Preservative Tube is not acceptable for urine chemistry testing.

Refer to the *Procedural Notes* section of this procedure for additional information on unacceptable specimens.

Specimen Storage and Stability

It has been shown that lactic acid may cause an increase in osmolality. Therefore, the specimen should be spun, and the serum separated from cells and refrigerated as soon as possible to reduce the rate of glycolysis and also to reduce the possible presence of particulate matter.

Urine should be collected in clean, dry containers without preservatives and centrifuged at sufficiently high speed to remove all gross particulate material. If the analysis cannot be carried out soon after collection, the specimen should be refrigerated. Before analysis, refrigerated specimens should be warmed to aid the complete solution of any precipitated substances.

Tubes of blood are to be kept closed at all times and in a vertical position. It is recommended that the serum or plasma be physically separated from contact with cells within two hours from the time of collection.

Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at 2°C to 8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

Sample Volume

Optimum volume for testing: 0.5 mL
Sample volume: 20 uL per test

Reagents

None.

Equipment

Advanced Micro-Osmometer, Model 3320.
Advanced Instruments, Inc., Two Technology Way,
Norwood, Massachusetts 02062.
800-225-4034. Fax 781-320-8181.

Advanced Instruments 20-µL Ease-Eject™ Sampler, part# **3M0825**

Advanced Instruments Micro sample Kit (500 tests):
Disposable sample cells; chamber cleaners, part# **3MA800**

Sampler Plunger Wires (2), part# **3M0828**

Replacement Sample Probe, part# **330700**

Calibration

Calibrator Required

Description	Part Number
50 mOsm/kg Calibration Standard (10 X 2mL ampules)	3MA005
850 mOsm/kg Calibration Standard (10 X 2mL ampules)	3MA085

Calibrator Preparation

None

Calibrator Storage and Stability

Osmolality Calibration Standards when stored unopened at room temperature are stable until the expiration date printed on the label. Once opened, calibrators should be parafilmed during calibration between samples, and discarded after successful calibration.

Calibration Information

The osmometer must have a valid calibration in memory before controls or patient samples can be run.

Under typical operating conditions, the osmometer must be calibrated every 500 tests, with certain parts replacement or maintenance procedures including plunger tip replacement, and whenever quality control values exceed acceptable limits with respect to the Clinical Chemistry Quality Control Procedure #3000T.

Calibration Procedure

1. When the display reads "Osmometer Ready", press the [NEXT] button until [CALIB] appears over the left button. Press it to initiate the calibration procedure. Calibration can be cancelled without changing the existing calibration by pressing the [EXIT] button. The display will briefly read "50 mOsm Calibration" and then prompt the user to insert a 50 mOsm calibration standard.
2. Follow the prompts on the instrument display. When the instrument completes the test and reports the result, remove the sampler and clean the cooling chamber. Continue testing 50 mOsm calibration standards until this calibration point is complete.
3. The calibration program will now briefly read "850 mOsm Calibration" and then prompt the user to insert an 850 mOsm calibration standard. Again, follow the prompts on the instrument display. Continue testing 850 mOsm calibration standards until this calibration point is complete.
4. Upon successful calibration, the instrument will briefly display "Calibration Complete", then “Osmometer Ready”.
5. Verify the calibration by running a Clinitrol™ 290 Reference Solution and controls, before testing patient samples.

Calibration Notes

Repeatability, or precision, is essential to calibration. A "calibration" is based on repeatable "hard" numbers for reference materials. Direct-reading calibration of the Advanced Instruments Model 3320 is a simple procedure that requires no adjustment of the instrument by the user. Simply run the menu-driven calibration program, which prompts the user to run a series of tests using calibration standards at 50 mOsm/kg H2O and 850 mOsm/kg H2O. When the tests are complete, the Advanced Instruments Model 3320 then calculates and stores the calibration coefficients.

The Model 3320 will retain its previous calibration data until it completes a new calibration, and the display reads "Calibration Complete".

If the instrument has calibration information in memory, the results displayed during the calibration procedure will be close to the nominal value of the standards used. If the instrument has no calibration information in memory, or if a probe has been replaced, the results displayed may be far from the nominal value of the standards used. If the displayed values repeat consistently, the calibration will automatically adjust when the calibration sequence is complete.

The calibration procedure may be terminated at any time by pressing [EXIT]. The instrument will display "Calibration Canceled", and beep twice. The previous calibration will be retained. The user will be prompted again to insert the appropriate calibration standard.

Quality Control

At least 3 levels of control material should be analyzed each shift. In addition, these controls should be run with each new calibration and after specific maintenance or troubleshooting procedures.

Description	Storage
MAS ChemTrak H Level 1 serum chemistry control	2° - 8°C*
MAS UrineChemTrak Level 2 urine chemistry control	2° - 8°C**
Advanced Instruments ClinitrolTM Reference Solution (10 X 2mL ampules, 290 mOsm/kg)	Room Temp***

*Controls are received frozen and stored at -15°C to -25°C. Bottles of controls in use are thawed and stored at 2°C to 8°C and are good for 14 days.
**Urine controls are received and stored at 2°C to 8°C. Bottles of controls in use are stored at 2°C to 8°C and are good for 30 days.
**Reference solution ampules are received and stored at room temperature and are stable until the expiration date stated on bottle.. Bottles of controls in use are aliquoted and stored at room temperature and are good for 30 days.

Testing Procedure

Osmometer Startup

The Osmometer will be always on for standby operation.

If Osmometer is off, turn the instrument on by pushing the rocker-style power switch on the instrument's back panel into the on (|) position. There will be a short delay until the instrument's display becomes active. Each time the power is turned on, the display provides critical information, such as software version and probe bin numbers. Record this information in the Service Log at the end of this User's Guide. Maintaining a record of this information will facilitate any service that may become necessary.

The user is then prompted to run a one-minute self-diagnostic test. It is highly recommended that the user runs this test. Bar graphs will appear, showing the block (B) and the sample (S) probe temperatures while the instrument cools down, and then while the instrument warms up.

When the self-diagnostic test has been completed, the display will read, "Osmometer Ready". Tests can now be run, or additional functions can be accessed from this display.

If Osmometer has been off, it should be allowed to stabilize at ambient temperature for 20 to 30 minutes before you run calibration verification tests, controls and patient samples.

Chamber cleaning

The cooling chamber and probe are easy to keep clean and dry by faithfully following the operating instructions for cleaning the freezing chamber after each test. If traces of calibrators, controls or biological samples are left in the sample chamber, however, the task will be more difficult and damp cleaning will probably be required. Two indicators that damp cleaning may be required are:

- The instrument has been in use but no clean, dry chamber cleaner is found in the sample port, and successive results on aliquots of the same sample indicate chamber contamination (the first aliquot reading is very high, and subsequent readings are progressively lower).
- "Sample Pre-freeze" errors begin to occur quite frequently.

When indicated, the cooling chamber may need to be damp cleaned. Refer to the Advanced® Micro-Osmometer Model 3320 User's Guide for instructions to perform this procedure.

Sample Test Procedure

1. Insert a sampler tip into place on the sampler. The sampler tip must be straight and firmly seated.
2. Depress the sampler's plunger and insert the sampler tip at least ¼ inch (6 mm) below the surface of the fluid to be tested. Gently release the plunger to load a 20-µL sample.
3. Visually inspect the sample. If there are any large voids or bubbles in the sample, expel the sample and load a bubble-free sample.
4. Wipe the sides of the loaded sampler tip with a soft, no-lint, nonionic paper tissue to remove any clinging droplets. Then quickly wipe the end of the sampler tip to remove any fluid protruding beyond the tip. Be careful not to remove any of the sample. The exposed surface of the sample must be level with the end of the tip or may be slightly concave. (See Figure 5, next page)

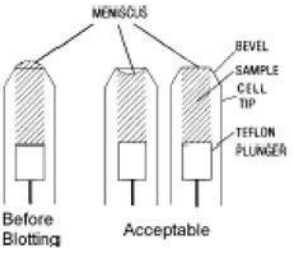


Figure 5: Sampler Tips and Sample Levels

5. Remove the chamber cleaner from the sample port and discard.
6. Holding the sampler by the barrel, insert the tip into the sample port, then rest the sampler in the operating cradle.
7. To start the test, push the operating cradle in until it reaches a positive stop. Your instrument will run the test for approximately one minute and display the result in the format “Osmolality xxx mOsm”. You may also start the test by pressing the left key on the keypad, and then pushing in the cradle.
   NOTE: To cancel a test in progress, use the same method used to start the test. If the cradle was used, pull back on the cradle. If the keypad was used, use the right “Cancel” key.
8. Record the results and pull back the operating cradle to a positive stop.
9. Remove the sampler from the operating cradle.
10. Insert a clean, dry chamber cleaner into the sample port and rotate it four or five times in both a clockwise and counterclockwise direction. Withdraw the chamber cleaner and insert the opposite end. Rotate the chamber cleaner in the same manner and leave it in the sample port until your next test. (See Chapter 5, Chamber cleaning in the Advanced® Micro-Osmometer Model 3320 User’s Guide.)
11. Remove the used sampler tip from the sampler by pressing firmly enough on the sampler plunger to dislodge the tip, or apply a slight bending force using the thumbs and forefingers where the tip is pressed onto the sampler. Discard the used sampler tip.
12. Wipe the Teflon plunger tip with a soft, no-lint, non-ionic paper tissue. Be careful not to dislodge the tip.

To run additional tests, repeat this procedure beginning with step 1. Report the average of two sequential determinations that agree within 2 mOsm/Kg.

Repeatability Tips

Treat all samples, as well as standards and reference solutions, uniformly before the test.

Micro-samples are more susceptible to contamination and evaporation than larger samples. Avoid leaving sample containers open. Cold samples are susceptible to condensation; warmer samples are susceptible to evaporation.

If an occasional sample produces irregular results, discard obviously discrepant readings as long as the instrument has been producing accurate readings with previous samples. Repeat the sample in question. Aliquot and re-centrifuge samples that continue to produce inconsistent results before repeating.

Only use the sampler either supplied with your instrument or listed in the parts and accessories section of this user’s guide. Each sampler comes with specific operating instructions and re-order information.

Cross-contamination from previous samples can affect the result obtained from a subsequent test. To minimize this effect when testing samples whose expected range is appreciably different from that for previous tests, run two or more replicates of the new sample and disregard the first result. Also, be sure to clean the cooling chamber and sampler properly after each test.

For repeat runs, use additional samples from the same source.

Proper cleaning of the cooling chamber between tests is very important. Follow the procedure provided in the maintenance binder. Never inject anything in cooling chamber.

Calculations

None.

Reporting Results

Reference Intervals

Sample Type	Reference Interval
Serum, Plasma	280 to 300 mOsm/Kg
Urine	300 to 1200 mOsm/Kg

Results are reported as “xxx mOsm/Kg”.

Values exceeding 1500 mOsm/Kg will be reported as “>1500 mOsm/Kg”.

Osmolality values are read directly from the display read-out of the instrument.

Patient results are averaged from 2 determinations and should be repeated until sequential results agree within 2 mOsm/Kg.

When results differ by 1 mOsm/Kg, report the “even” whole number result. (Round to make even.)

Critical Values

None.

Procedural Notes

Samples and standards should be tested at room temperature.

Samples may be re-tested in original tubes after they have reached room temperature. However, evaporation must be safeguarded against.

Never sample standards directly from the container. Pour a small aliquot into a test tube before sampling. Keep standard bottles tightly capped.

Do not dilute standards as the activity coefficient varies with concentration. The freezing point is not linearly related to the amount of dilution.

Conversion of all osmolality values to a per kilogram solvent water basis has the advantage of permitting direct comparison of such divergent body fluids as serum and water. Serum, for example, has only 90 to 93% water content.

The average freezing point of normal serum is -0.53 C. Since a 1000 mOsm/kg solution depresses the freezing point of water 1.86 C, the determined osmolality of serum is: mOsm/kg serum H2O = 1000 x 0.53/1.86 = 285 mOsm.

Many ionic compounds in serum dissociate less than 100%. Some ions are bi- or trivalent. There are negative influences of proteins on colligative properties of ions in solution to consider as well as numerous complex inter-ionic depressing effects.

Sodium and its salt with other electrolytes (chloride and $HCO3^-$) account for about 275 mOsm/kg serum water. Non-ionized organic compounds, principally glucose and urea, add an additional 10 mOsm/kg. Protein is of large molecular size and contributes little (1-4 mOsm/kg).

The normal ratio between serum sodium concentration and osmolality is:

$$\frac{\text{mEq/L sodium}}{\text{Osmolality}} = 0.43 \text{ to } 0.50$$

In hyponatremia without an increase in other serum solutes, the ratio should remain normal. If other serum solutes are increased (as in hypernatremic renal failure), the ratio is reduced, which is indicative of a poor prognosis.

Osmolality may be predicted from serum concentration of sodium, urea nitrogen, and glucose. Two of the most common equations are listed here. (7)

$$\text{Calculated Osmolality} = 1.86[\text{Na}] + \frac{[\text{glucose}]}{18} + \frac{[\text{BUN}]}{2.8}$$

$$\text{Calculated Osmolality} = 1.86[\text{Na}] + \frac{[\text{glucose}]}{18} + \frac{[\text{BUN}]}{2.8} + 9$$

Differences between measured and calculated values higher than 40 mOsm/Kg indicate a poor prognosis.

Osmolality may be approximated by doubling the sodium and adding 7.

Normally the ratio urine osmolality/serum osmolality should exceed 1.0; after an overnight fast the ratio should be 3.0 or greater. It becomes equal to 1.0 in chronic renal disease. It is less than 1.0 in diabetes insipidus or excretion of a large water load.

Osmolality is a better measurement than specific gravity in gauging the concentration ability of the kidney, which responds physiologically to changes in the concentration of particles and not to their weight. The specific gravity of urine depends upon the weight of its solutes, rather than the particle concentration.

Heavy molecules like glucose and albumin will influence the specific gravity out of proportion to their relative concentration in the urine, but do not affect osmolality to the same degree.

Although the correlation between specific gravity and osmolality may be good in normal subjects with low urine solute concentration, this correlation becomes quite poor when there is a high solute because a greater proportion of the solute will consist of urea and other non-ionized compounds. A specific gravity of 1.020 may represent variation in osmolality from 550 to 900 mOsm/kg.

Known Interferences

Viscous solutions.

Samples containing undissolved particles in suspension or precipitate may cause pre-seeding and/or pre-freeze errors which may be eliminated by warming or centrifuging the sample.

Hyperlipemia may cause interference with uniform freezing of sample.

Hemolysis seems to increase osmolality only slightly.

Drugs and anesthetics may increase serum osmolality either due to dehydration or as a direct result of the treatment. Diuretics may have the same effect on serum while diluting urine

Performance Characteristics

Analytical Measurement Range

0 to 1500 mOsm/Kg

Clinical Reportable Range:

0 to 1500 mOsm/Kg

Values exceeding 1500 mOsm/Kg should be reported as ">1500 mOsm/Kg".

Linearity Verification

Two osmolality standards (900 and 1500 mOsm/Kg) were diluted 1:2, 1:3, 1:4, and 1:5 with deionized water and assayed for linearity and recovery.

Standard (mOsm/Kg)	Dilution	Observed (average) (mOsm/Kg)	Expected Value (mOsm/Kg)	Recovery (%)
900	---	899	900	99.9
	1:2	446	450	99.1
	1:3	298	300	99.3
	1.4	222	225	98.7
	1:5	175	180	97.2
	mean			98.8
1500	---	1503	1500	100.2
	1:2	748	750	99.7
	1:3	490	500	98.0
	1:4	372	375	99.2
	1:5	292	300	97.3
	mean			98.9

Precision

Precision specifications from Advanced Instruments are as follows:

± 2 mOsm/kg H2O (1 S.D.) between 0 and 400 mOsm/kg H2O

± 0.5% (1 S.D.) between 400 and 2000 mOsm/kg H2O

Precision determined at UCDMC:

Type of Precision	Sample Type	Mean mOsm/Kg	1 SD mOsm/Kg	%CV
Within-Run	ChemTrak 1	439	2.6	0.6
Day to Day	ChemTrak 1	442	4.5	1.0
Total	ChemTrak 1	441		1.18
Within-Run	ClinTrol 290 STD	291	1.3	0.5
Day to Day	ClinTrol 290 STD	292	2.1	0.7
Total	ClinTrol 290 STD	292		0.85
Within-Run	900 STD	905	2.6	0.3
Day to Day	900 STD	908	4.2	0.5
Total	900 STD	907		0.54

Drift

Less than 1 mOsm/Kg H_2O per month.

Performance at Operating Conditions

Temperature effects: less than 1 mOsm/Kg H_2O per 5°C ambient temperature range.

Accuracy

Clinical measurement of osmolality is accomplished by indirect means as direct measurement of osmotic pressure is far too cumbersome to be practical. Two methods are currently popular: freezing point depression and vapor pressure depression. The methods correlate very well on aqueous biological fluids with the freezing pint method showing no interferences from volatiles.

The following correlation results are from comparing the current Osmometer (Model 3300) in use with the new Osmometer (Model 3320) from Advanced Instruments.

Equivalency was assessed by Deming regression analysis of patient samples to accepted clinical methods as determined at UCDMC.

Serum or Plasma (osmolality in the range of 88 to 688 mosm/kg):

Y (Model 3320)	= 0.997X - 2.5
N	= 63
MEAN (Model 3320)	= 311
MEAN (Model 3300)	= 315
CORRELATION COEFFICIENT (r)	= 0.9994

Urine (osmolality in the range of 180 to 1194 mosm/kg):

Y (Model 3320)	= 1.003X - 6.5
N	= 40
MEAN (Model 3320)	= 571
MEAN (Model 3300)	= 575
CORRELATION COEFFICIENT (r)	= 0.9997

Errors

Occasionally a test will not run to completion and the instrument will display an error message. Refer to the Troubleshooting Table in Appendix A of the *Advanced Micro-Osmometer Users Guide*, or refer to Chapter 5 of the Users Guide for Additional Troubleshooting and Service information.

Sample Plunger Wire replacement and Verification

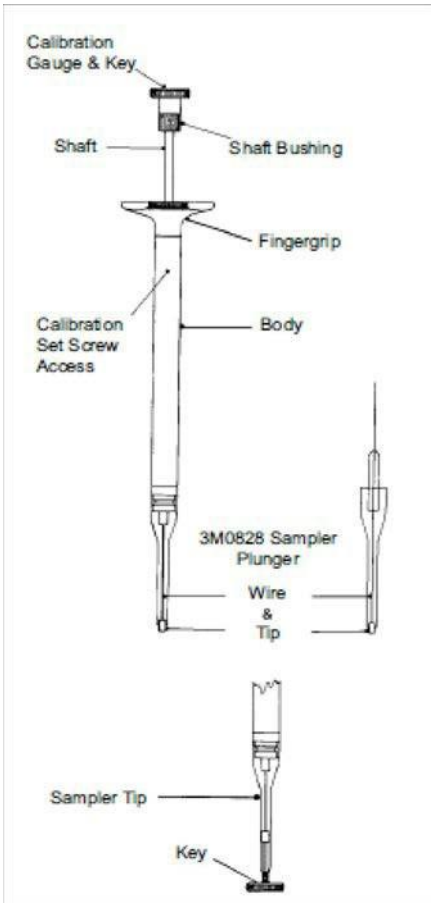
To ensure proper instrument operation, you should replace the plunger wire tip of the sampler every 500 tests (or every package of sampler tips).

Calibration must be performed after sample plunger wire replacement.

To replace the plunger tip, use the following procedure. (*Refer to image on next page*.)

1. Unscrew the calibration gauge and key.
2. Rotate the sampler shaft until the calibration setscrew appears beneath the access hole in the side of the sampler body.
3. Place the key end of the calibration gauge in the access hole and turn counter-clockwise to loosen the setscrew.
4. Carefully remove the old sampler plunger wire.

5. Place a sampler tip on the sampler to help you place new wire correctly. Slip the sampler plunger wire into the sampler tip so the Teflon plunger tip protrudes about 1/16” or 1.6 mm from the end of the sampler tip.
6. Using the key end of the calibration gauge, push the plunger wire into the sampler as far as it will go.
7. Tighten the calibration setscrew with the calibration gauge.
8. Screw the calibration gauge and key back into the top of the sampler. The 20-µL sampler is now ready to use.
9. Calibrate the Micro-Osmometer and run controls to verify acceptable performance.



Sample Plunger Wire Replacement

Additional Information

For more detailed information on the Advanced Micro-Osmometer, refer to the *Advanced Micro-Osmometer Users Guide*.

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Prepared By	Date Adopted	Supersedes Procedure #
M. Inn	08/28/2010	Osmometer Model 3300

Revision Date	Type of Revision	Revised by	Review/Annual Review Date	Reviewed By
			08/27/2010	S. Deveraj
06/28/2011	Removed sample type Fluids	M. Inn	11/16/2011	G. Kost
04/09/2014	general update	M. Inn		
07/18/2014	sample replicate agreement change	kdagang	07/24/2014	J. Gregg
04/03/2015	Replace 900 STD with MAS Urine Control	kdagang	07/24/2015	J. Gregg
			08/28/2015	J. Gregg
12/31/2016	clarified calibration required after plunger tip replacement	kdagang		