**1.0 PRINCIPLE**

Osmometry is a technique for measuring the concentration of solute particles. When solute is dissolved in a pure solvent, the following properties of the solvent are changed:

 1. The freezing point is depressed

 2. The boiling point is raised

 3. The osmotic pressure is increased

 4. The vapor pressure is lowered

 These are the so-called colligative or concentration properties of the solvent and, within reasonable limits, change in direct proportion to the solute concentration; in other words, the number of particles in solution. The proportionality constant is characteristic of the particular solvent. For water, one gram/mole (Avogadro's number) of non-ionizing solute dissolved in one kilogram of water will depress the freezing point by 1.858 degrees C. If solute does dissociate, then all ionized and non-dissociated species must be counted.

 Freezing Point in osmometry, is that unique temperature, at atmospheric pressure, at which the solid and liquid phases will co-exist in equilibrium. When a solute is dissolved in pure solvent the colligative or concentrative properties of the solvent change, within reasonable limits, in direct proportion to the solute concentration. Of these properties, measurement of the freezing point, allows the concentrations to be determined with the greatest precision because inherent isolation of the sample from the environment is provided in the ice blanket generated when the sample freezes.

 The quickest and most precise way to measure the freezing point of a solution is to cool it several degrees below its freezing point. When the sample is introduced, it goes through a fast cool. Once the sample hits 0 degrees C., the cooling of sample slows. When the sample has been cooled a mechanical pulse induces the rise toward a plateau wherein ice/water equilibrium is maintained. As soon as the plateau is constant, the sample osmolality is locked and display reads.

**2.0 PURPOSE**

This procedure is intended to provide instructions and guidelines for performing osmolality using the Advanced Micro-Osmometer Model #3320 **(EMC-P)** and Advanced Micro-Osmometer Model #3300 **(EMC-EP)**

**3.0 SPECIMEN REQUIREMENTS**

Serum: 0.1 ml - serum/plasma (Heparin) stable up to 3 hrs at room temp, after 3 hrs- refrigerate at 1-6 degrees C for 24 hrs

Urine : 0.1 ml – random, no preservative needed / 24hr , no preservative needed. Centrifuge urine to remove all gross particulate material. If the analysis cannot be done in 3 hrs, refrigerate at 1-6 degrees C for 24 hrs.

**4.0 MATERIALS AND EQUIPMENT REQUIRED**

4.1 Standards:

50 mOsm/kg Calibration Standard (Cat #3MA005, Advanced Instruments, Inc.)

850 mOsm/kg Calibration Standard (Cat #3MA085, Advanced Instruments, Inc.)

Clinitrol Reference Solution 290 mOsm/kg (Cat #3MA029, Advanced Instruments, Inc.)

4.2 Controls:

Bio-Rad Liquichek Unassayed Chemistry Control Levels 1 and 2 **(EMCP-EMC-EP)**

Bio-Rad Liquichek Urine Chemistry Control Level 1 and 2 **(EMCP)**

MAS Urine Control Level 1 and 3 **(EMCP-EP)**

4.3 Calibration verification:

5-Value Osmolality Linearity Set (Cat#3LAO28, Advanced Instruments, Inc)

4.4 Supplies:

Micro-Sampler Kit: 500 disposable chamber cleaner and 500 sampler tips (Cat# 3MA800, Advanced Instruments, Inc)

20 microliter Ease-Eject Sampler (Cat #3M0825, Advanced Instruments, Inc)

Kim-Wipes (Cat# 3455, Kimberly-Clark)

1. **CALIBRATION**
	1. Calibration is required:
		1. At least every 6 months
		2. When requested by instrument
		3. When 290 mOsm/kg standard is out of range
		4. When controls fall outside of acceptable range
		5. After major maintenance is performed. (i.e. Plunger Wire Replacement, see 7.5 for instructions on replacement of sample plunger wire.)
	2. Calibration is menu driven, prompting the user to run a series of tests that measures reproducible results at the 50 mOsm level and at the 850 mOsm level. When the testing is complete, the Advanced Instrument model #3320/#3300 then calculates and stores the calibration coefficients.
	3. Calibration Procedure:

5.3.1 When display reads “Osmometer Ready”, press [next] button until [calib] appears over the left button. Press to initiate the calibration procedure.

* + 1. Display will show “50 mOsm calibration”. Clean the chamber with chamber cleaner (white straw) by rotating two or three times with clean end of white straw. Draw up the 50mOsm standard in sample syringe (20ul). Wipe all excess sample from the outside of the sample tip with a clean, no-lint, paper tissue (Kim-Wipes). Insert syringe. Press start.
		2. Run the 50 standard until the display reads “850 mOsm Calibration”. The process will repeat 2-3 times before the instrument accepts the calibration. After each repeat the old tip is discarded, replaced with new tip, and excess from the outside of sample tip is wiped.
		3. Following the procedure technique, when display reads “850 mOsm Calibration”, clean the chamber , and draw up the 850mOsm standard in the sample tip, wipe, and insert syringe. Repeat the process with this standard, until display reads “Calibration Complete”.
		4. After completing, run Clinitrol 290 mOsm/kg reference solution. The range is 290 mOsm/kg ± 2. Verify calibration by running urine and serum QC specimens. Document QC in the Osmolality log book and in computer. Document calibration on the Osmometer QC log.
	1. Calibration Verification

5.4.1 Used to verify the analytical measurement range (AMR) of the osmometer semi-annually.

5.4.2 Run the 5-value Osmolality Linearity set (10 x 5ml ampules, 2 of each value: 100, 500, 900, 1500 and 2000 mOsm/kg) in triplicate.

* + 1. Procedure: Carefully snap off the top of the ampule. Follow proper sampling technique and obtain samples using a new sample tip for each test. Run each level three times. Average the three results and plot on a linearity curve.
		2. Expected values: 50 mOsm/kg-- 48-52 mOsm/kg

 100 mOsm/kg—98-102 mOsm/kg

 500 mOsm/kg—497.5-502.5 m0sm/kg

 900 mOsm/kg—895.5-904.5 m0sm/kg

 1500 mOsm/kg—1492.5-1507.5 mOsm/kg

 2000 mOsm/kg—1990-2010 mOsm/kg

5.4.5 Interpretation-Osmometer results are evaluated with respect to the total expected range, which combines the effects of the osmometer standard expected range. If results fall outside the total expected range, it may indicate unsatisfactory calibration, operator error, contamination of reagents , or faulty performance of equipment.

1. **QUALITY CONTROL**
	1. Run two levels of appropriate controls on each osmometer once daily (EMCP). Run two levels of appropriate controls as per test request, once per calendar day at(**EMC-EP**). Controls are run after calibration or after any service procedure and record on **CH04-001 Form A1** and **CH04-001 Form B2 (EMCP)** and **CH04-001 Form C1 (EMC-EP),**  **CH04-001 Form D1** **(EMC-EP) and results are entered into Cerner .**
		1. Run Biorad Unassayed Serum Level 1 and 2 on the Osmometer named **Osmo (EMCP only)**
		2. Run Biorad Urine Chemistry Level 1 and 2 on the Osmometer name **Osmo2. (EMCP only)**
		3. Run Biorad Unassayed Serum Level 1 and 2 on Osmometer as per test request. **(EMC-EP only)**
		4. Run MAS Urine Controls Level 1 and 3 on Osmometer as per test request. **(EMC-EP only)**
		5. Note: When an osmometer is removed from service, run both levels of each control, serum and urine, on the remaining osmometer in use (**EMCP**).
	2. Run the 290 mOsm/kg reference solution once per shift on both instruments(**EMC-P**) or as per test request at (**EMC-EP**) . **Note**: 290 reference solution must be performed on each shift as per test request record results on CH04-001 Form C and CH04-001 Form D at (**EMC-EP).** **Results will only be entered on manual log and not entered into Cerner for the 290 STD.**

6.3 Run controls in the same manner as patient samples. The run is acceptable if one control is within ± 2SD, and alternate control is between 2-3 SD. If neither control is within ± 2 SD, calibrate and repeat analysis.

**7.0 *EASE-EJECT* ™ 20-µL SAMPLER OPERATION**

7.1 Sampler Tip installation

7.1.2 Slide one sample tip over plunger wire and mount firmly on the sampler body. Be sure the sampler tip is straight and seated tightly.

 7.2. Sampling

7.2.1 Depress the plunger knob and insert the sample tip into the sample at least ¼ below the fluid surface.

7.2.2 Gently release the plunger to draw 20-ul of sample into sample tip and remove the sampler from the sample.
7.2.3 Visually inspect the sample for any large voids or bubbles. If any, expel and redraw sample.

7.2.4 Wipe and/or blot all excess sample from the outside of sample tip with a clean, no-lint, nonionic paper tissue to remove any clinging droplets. But be careful, not to wick out the sample. The sample should not extend beyond the end of the sample tip. If you are in doubt, leave a slightly concave meniscus. Use a fresh sample tip for each sample.

7. 3 Sample Tip removal- Press down on plunger knob or apply a slight bending force using the thumb and forefinger where tip is pressed onto sampler to dislodge the tip. Remove it from the plunger wire with a lint-free tissue to avoid contaminating the next sample.

7.4 Between runs, rinse the plunger tip with water or alcohol and wipe dry.

7.5 Replace the sampler plunger wire with each new test kit (500 samples).

* 1. Plunger Wire replacement:
		1. Unscrew the calibration gauge and key

7.6.2 Rotate the shaft until the calibration setscrew appears beneath the access hole in the side of sampler body.

* + 1. Use the calibration key to loosen the setscrew.

7.6.4 Carefully remove the old sampler plunger wire. If it contains a plastic sleeve, save and install on the new plunger.

* + 1. Slip a new sampler plunger wire into a new sample tip so the Teflon plunger tip protrudes about 1/16” or 1.6mm.
		2. Using the calibration gauge and key, push the plunger into sampler as far as it will go.
		3. Tighten the calibration setscrew with the calibration gauge.
		4. Screw the calibration gauge and key back into the top of the sampler.
		5. The sampler is now calibrated and ready to go.

 7.7 Sampler Calibration

 For calibration only, follow steps 1, 2, 3, 6, 7 and 8 above.

**8.0 SAMPLE TEST PROCEDURE**

* 1. Insert a sampler tip into place on the sampler. The sampler tip must be straight and firmly seated.
	2. Acquire sample following proper sampling technique. Visually inspect sample.
	3. Wipe off excess sample of the loaded sampler tip with a soft, no-lint paper tissue (Kim Wipes).

8.4 Remove the chamber cleaner from the sample port and discard

8.5 Holding the sampler by the barrel, insert the tip into the sample port, then rest the sampler in the operating cradle.

8.6 To start test, push the operating cradle in until it reaches a positive stop. The instrument will run the test for approximately one minute and display the result in the format “Osmolality xxx mOsm”.

8.7 Record results in the stat lab patient log sheets and pull back the operating cradle to a positive stop

8.8 Remove the sampler from the operating cradle and insert a clean, dry chamber cleaner into the sample port and rotate a few times

8.9 Withdraw the chamber cleaner and insert the opposite end to leave in sample port until next test. Proper cleaning of the cooling chamber between tests is very important. Never inject anything into the cooling chamber.

8.9.1 Record results on patient test logs CH04-001 Form E and CH04-001 Form F (EMC-EP) and CH04-002 Form C2 at EMCP and Enter results in laboratory computer.

**9.0 Reference Range**

 Urine: 300-1090 mOsm/kg

 Serum: 270-310 mOsm/kg

**10.0 Critical Values:**

**CV Low:** Serum Osmolality less than 250 mOsm/kg

**CV High:** Serum Osmolality equal to or greater than 335 mOsm/kg

**10.0 Calculations: N/A**

**11.0 LIMITATIONS OF PROCEDURE**

1. Serum osmolality should be approximately twice the serum Na+ value.
2. Linearity: 0-2000 mOsm/kg H2O. Linearity is verified semi-annually. Report samples that test greater than 2000 as “Greater than 2000”.
3. Heparinized plasma samples can be substituted for serum, but other plasma anticoagulants may add measurable solute to the blood and make interpretation difficult.
4. **REFERENCES**

Advanced Micro-Osmometer Model 3320 Users’ Guide, 2005.

Kaplan, *et al, Clinical Chemistry: Theory, Analysis and Correlation*, 3rd ed., 1996.

Tilton, *et al*, Clinical Laboratory Medicine, 1st ed., 1992.

**Approval Signatures:**

|  |  |  |
| --- | --- | --- |
| **Date** | **Printed Name** | **Signature** |
| 7/16/14 | Jennifer Lore, MFS, MTChemistry Supervisor | N/A – New Leadership Change |
| 2/10/2016 | Vanessa Rawlings, MHA, MTElkins Park Supervisor |  |
| 7/16/14 | Nancy A. Young, M.D., FCAPMedical Director | N/A – New Leadership Change |

**Review History**

|  |  |  |
| --- | --- | --- |
| **Date Reviewed** | **Reviewed By** | **Revisions** |
| 7/30/2015 | J. Lore/ T. Cameron | Added minor example to 5.1.5 of what is considered major maintenance.  |
| 2/10/2016 | J. Lore/ V. Rawlings | Updated EMCP Patient form to CH04-002 Form C2.  |
| 9/14/2016 | J. Lore/ V. Rawlings | Added details to entering of QC, minor revision.  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |
|  |  |  |