Cone Health Laboratories

TITLE: ADVANCED® INSTRUMENTS MICRO-OSMOMETER OSMOLALITY BASIC OPERATING PROCEDURE

PRINCIPLE / PURPOSE: The osmometer provides results that assist in establishing the proper diagnosis and treatments for patients with disorders involving water and electrolyte imbalances and the determination of osmolality can be useful in the assessment of electrolyte and acid/base disorders.

When a solute is dissolved in a pure solvent, the following changes in the properties of the solvent occur.

- The freezing point is depressed.
- The boiling point is raised.
- The osmotic pressure is increased.
- The vapor pressure is lowered.

These are the so called colligative or concentrative properties of the solvent which, within reasonable limits, change in direct proportion to the solute concentration-the number of particles in solution. The term osmolality is used to express the reactive osmotic pressure of a solution in terms of mass of solute per mass of solvent. Of the colligative properties, measurement of the freezing point, where applicable, allows the concentration of the solution to be easily determined with great precision.

Osmolality is a common unit of concentration measurement that can be used to relate all the colligative properties to each other, and to other concentrations units. Because of its university, most osmometry applications regularly use osmolality, expressed as "mOsm/kg H2O", as the common unit of concentration rather that applying further conversion factors.

SCOPE: This document relates to testing a patient's specimen for osmolality on the Advanced Instruments Micro-Osmometers in the Cone Health Clinical Laboratories.

COMPLEXITY LEVEL: Moderate

SAFETY:

- The required personal protective equipment for this procedure
  - Gloves
  - Approved lab coats, worn closed
- Gloves and lab coats should be worn at all times during analysis of the samples.

SPECIMEN:

Specimen Type:

Serum or *Plasma: (Plain red, SST, *lithium heparin) Collect blood by venipuncture, with a minimum of stasis. Allow serum samples to completely clot before separating the serum by centrifugation.

**Plasma option only available at the ARMC location*

Urine: Collect urine in clean, dry, capped containers without preservatives. Centrifuge all urine in a clean pour off tube in the chemistry centrifuge to remove gross particulate matter.

Volume Required: 20 µL of sample is required for each test to be performed.

Stability and Storage:

Serum and Plasma: Stable for 24 hours at room temperature and three days refrigerated at 2°-8° C. (Plasma only validated at ARMC)

Urine: Stable for 8 hours at room temperature and refrigerated 24 hours at 2°-8° C.

Unacceptable specimens:

- Gross hemolysis
- Excessive lipemia, improperly labeled specimen, inappropriate specimen type.

EQUIPMENT AND MATERIALS:

Advanced Instruments Micro-Osmometer
Model 3320 located at Moses Cone
Model Osmo1 located at ARMC
Quality Controls
Advanced Instruments Ease-Eject Sampler (20 uL)
Advanced Instruments Sampler Tips
Advanced Instruments Chamber Cleaners
Thermal Paper

MAINTENANCE:

Daily:

Clean the instrument exterior: Clean the instrument exterior with isopropyl alcohol prep pads. **DO NOT** use abrasive cleaners or scouring pads, as they may damage the surface. **DO NOT** allow liquid to enter the cooling chamber or other portions of the instrument.

Weekly:

Clean the Air Vent: Examine the air vents of the instrument to ensure that it is unobstructed by dust or debris.
Model 3320 has an air vents on the underside and rear of the instrument
Osmo1 instrument has air vents on the rear of the instrument only

Monthly:

Clean the Solenoid Impactor: Clean monthly. See the Advanced® Micro-Osmometer User's Guide for complete instructions on how to properly perform cleaning.

As Needed:

Fuse Replacement: See the Advanced® Micro-Osmometer User's Guide for complete instructions on how to properly perform task.

Osmo1 Model has additional as needed requirements:

Chamber Cleaning: If you experience multiple Sample Pre-freeze errors, or if you suspect contamination of the sample probe, clean the cooling chamber with a chamber cleaner that has been dampened with water.

Sample plunger wire replacement and verification: Replace the sample plunger wire every 500 tests (or each time you empty a Micro-Sample Test Kit). See the Advanced® Micro-Osmometer User's Guide for complete instructions on how to properly perform task.

CALIBRATION:

Calibrators/Standards: Advanced Instruments Osmometer Standards and Controls are supplied ready to use, and require no preparation other than mixing by gentle swirling prior to use.

Storage and Stability:

Reagent Name:	Storage Temperature	Stability
Advanced Instruments Calibration Standards	2 - 30° C	**Unopened**: Stable until the expiration date printed on the label when stored at 2 - 30° C. **Opened**: Expires at the end of the testing day
5-Value Osmolality Linearity Set:	2 - 30° C	**Unopened:** Stable until the expiration date printed on the label when stored at 2 - 30° C. **Opened:** Expires at the end of the testing day
Clinitrol™ 290 Reference Solution:	2 - 30° C	**Unopened:** Stable until the expiration date printed on the label when stored at 2 - 30 °C. **Opened:** Expires at the end of the testing day

Standard Preparation: No special preparation required. Standards are ready to use on arrival from Advanced Instruments.

Calibration Frequency:

It has been shown that unnecessary recalibration will introduce inaccuracy. The calibration of a freezing point osmometer should not shift or drift. The Advanced® Osmometer must be calibrated:

- At the initial set up of the analyzer
- If the test results for the reference solutions are out of specification
- If the ambient temperature has changed more than 5°C since the last calibration
- After re-setting the block or sample pin numbers
- If calibration data is lost
- If QC fails to meet established criteria
- After major maintenance or service
- When operator wishes for troubleshooting
- At least every 6 months

NOTE: If calibration fails, repeat calibration. If the calibration fails again, contact Advanced Instruments Technical Support for further assistance.

Model 3320 Osmometer calibration procedure begins page 5

A. OSMO1 Calibration Procedure:

**Once prompting the analyzer to begin a calibration sequence – ALWAYS FOLLOW THE DISPLAYED PROMPTS. Calibrate the analyzer starting at the LOW RANGE.

Calibration NOTE: The ambient air may contaminate or evaporate samples left in open containers for any appreciable amount of time before testing.

In an extremely dry environment, high-range osmometer standards will evaporate and increase in osmolality very rapidly.

1. From the Home screen, tap the menu icon (Figure 22).

Figure 22: Menu icon

The Main menu displays (figure 23).

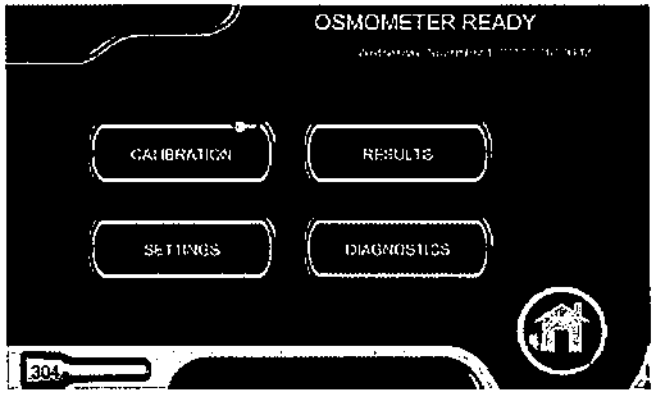

Figure 23: Osmol Main menu

2. From the Main menu, tap Calibration. The system prompts you to log in.
3. Select "Admin" from the list of available users.
4. Enter the password and tap Enter. The Calibration screen displays (Figure 24).

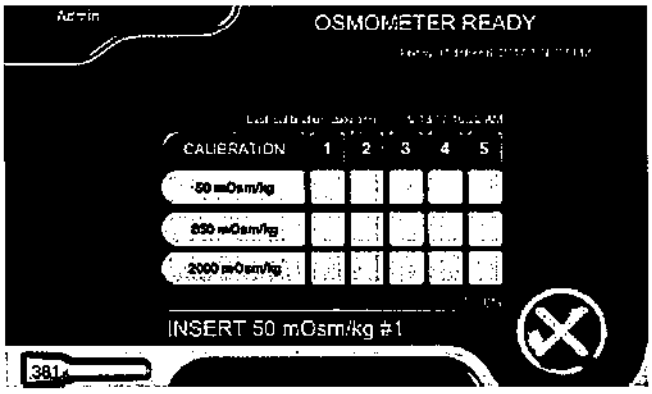

Figure 24: Calibration screen (before beginning Calibration procedure)

5. Follow the on-screen instructions to test samples from each specified standard five times.

a. After each successful calibration test, a green checkmark appears in the calibration matrix (Figure 25) and the unit prints "DONE" for the calibration test.

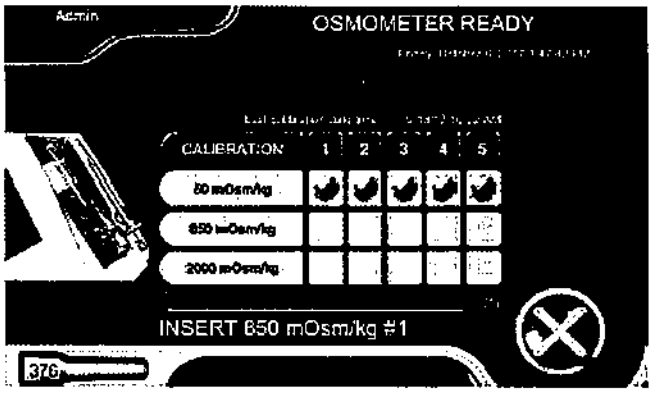

Figure 25: Calibration screen (calibration in progress)

b. If a single calibration test fails or is canceled, the system prompts for a retest using a new sample.
c. If two failures occur within the same standard group, that calibration fails and the screen displays the message "Two replicate Failures".

Figure 26: "Two replicate failures" error message

d. Upon completion of the last calibration test, the system displays a "Calibration successful" message (Figure 27) or the reason for failure.

Figure 27: "Calibration successful" message

6. Click OK to close the success message (or failure) message.
   a. When you close a success message, the system returns you to the Home screen.
   b. When you close a failure message, the system clears all checkmarks and returns you to the Calibration screen (Figure 24). From there, you can restart the calibration or exit to the Home screen.
7. Verify the calibration by running the Clinitrol 290 Reference Solution twice before performing patient samples.

B. 3320 Osmometer Calibration Procedure:

**Once prompting the analyzer to begin a calibration sequence – ALWAYS FOLLOW THE DISPLAYED PROMPTS. Calibrate the analyzer starting at the LOW RANGE.

Calibration NOTE: The ambient air may contaminate or evaporate samples left in open containers for any appreciable amount of time before testing.

In an extremely dry environment, high-range osmometer standards will evaporate and increase in osmolality very rapidly.

1. At "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.
2. Display will briefly read **"50 mOSM Calibration"** and then prompt the user to insert a 50 mOsm calibration standard. 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 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. Follow the prompts on the instrument display. Continue testing until this calibration point is complete.
4. The calibration program will now briefly read **"2000 mOsm Calibration"** and then prompt the user to insert an 2000 mOsm calibration standard. Follow the prompts on the instrument display. Continue testing until this calibration point is complete.
5. Upon successful calibration, the instrument will briefly display **"Calibration Complete"**, then **"Osmometer Ready"**. Attach the print-out sheet the that month's maintenance log.
6. Verify the calibration by running the Clinitrol 290 Reference Solution twice before performing patient samples.

LINEARITY:

The Advanced® Linearity Set contains 5 levels of NaCl standards (100, 500, 900, 1500 and 2000 mOsm). This matrix appropriate material spans the low, mid, and high range of the analytic measurement range and is used to verify the reportable range of patient test results. This must be performed:

- Following changes in major system components

Follow the steps outlined in the Calibration, Calibration Verification, Analytical Measurement Range and Clinical Reportable Range Procedure.

QUALITY CONTROL:

A. Clinitrol™ 290 Reference Solution

- Run once each day prior to QC testing to check repeatability, accuracy, instrument operation and confirm calibration.
- The user will also run Clinitrol™ 290 Reference Solution when erratic results are obtained. This will allow the user to verify proper operation of the instrument,or allow the user to recognize and correct problems promptly.

The Clinitrol™ 290 Reference Solution is tested a minimum of 2 times as per the sample test procedure.
Accuracy is demonstrated when all the sample results are between 288 and 292 mOsm/kg H_2O.
Precision (Repeatability) is demonstrated when the two sample results are ±2 mOsm/kg H_2O.
If the result is out of specification, retest a different sample vial of Clinitrol™ 290 Reference Solution. If the second result is out of specification, the instrument must be recalibrated as per the procedure outlined in the CALIBRATION section of this document.

NOTE: To identify sample carryover controls will be ran from highest value to lowest value.

B. Protinol® 3-Level Control Kit

Run once per day. Protinol protein-based controls provide control values that bracket values of most serum samples. These controls are formulated to mimic the freezing characteristics of actual serum samples and can be used to verify the precision and reliability of the osmometer results.
Protinol® Controls are tested as per the sample test procedure outlined under the Procedure section.

If the result is out of specification, test another sample of Protinol Control to determine whether the result is due to insufficient sample chamber cleaning or sample carryover.
If the second result is out of specification, test a sample of Clinitrol™ 290 Reference Solution to determine whether the instrument calibration is valid. If the Clinitrol™ 290 Reference Solution result is out of specification, recalibrate.

C. Renol™ Urine Osmolality Controls

Run once per day. Renol 2-Level Urine Osmolality Controls are formulated with urea to provide a "like-matrix" to urine samples, and control values that bracket most urine samples. These controls are formulated to mimic the freezing characteristics of actual urine samples and can be used to verify the precision and reliability of osmometer results.

Renol Controls are tested as per the sample test procedure outlined under Procedure section.

If the result is out of specification, test another sample of Renol Control to determine whether the result is due to insufficient sample chamber cleaning or sample carryover. If the second result is out of specification, test a sample of Clinitrol™ 290 Reference Solution to determine whether the instrument calibration is valid. If the Clinitrol™ 290 Reference Solution result is out of specification, recalibrate.

Record QC results.

- ARMC staff record results on the Osmometer clipboard.
- Moses Cone staff enter QC into the LIS under worksheet MCUA3 using the following QC Codes:

TEXT CODE	TRANSLATION
OCC290	OSMO QC 290
OCS240	OSMO SER QC 240
OCS280	OSMO SER QC 280
OCS320	SOMA SER QC 320
OCU300	OSMO UR QC 300
OCU800	OSMO UR QC 800

PROCEDURE:
Procedure for 3320 Osmometer begins on page 10

A. Procedure for Osmo1 model Osmometer:

1. From the Home screen check the status indicator and take the appropriate action.
   a. Green (Osmometer Ready): Go to Step 4.
   b. Orange (IDs Required): Go to Step 4.
   c. Yellow (Testing in progress): Wait for the current test to complete.
   d. Red (error condition): Refer to "Troubleshooting" on page 59 of the Operator's Manual.
2. Tap the Sample ID button. A keyboard displays and the barcode scanner on the front of the instrument activates.
3. Either type the container identification number or Scan the Sunquest patient label. CID) will appear on the instrument display and print-out after testing is complete.
4. Insert a sampler tip into place on the sampler. The sampler tip must be straight and firmly seated.
5. Depress the sampler's plunger and insert the sampler tip at least ¼ inch (6mm) below the surface of the fluid to be tested. Gently release the plunger to load a 20-uL sample.
6. Visually inspect the sample. If there are any large voids or bubbles in the sample, expel the sample and load a bubble-free sample.
7. Wipe the sides of the loaded sampler tip with a soft, no-lint, non-ionic 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 remove any of the sample. The exposed surface of the sample must level with the end of the tip or may be slightly concave.

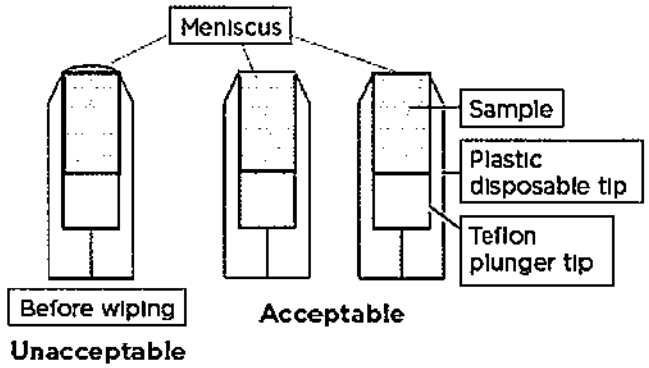

Figure 17: Sample tips and levels

8. Remove the chamber cleaner from the sample port and discard.
9. Scan Sunquest patient label before testing. (Holding the sampler by the barrel, insert the tip into the sample port, then rest the sampler in the operating cradle.
10. To start the test, push the operating cradle in until it reaches a positive stop. It will take approximately one minute to perform testing. When the test begins, the progress bar on the screen begins to fill. The status bar at the top of the display is yellow and status is TESTING. NOTE: to cancel a test in progress, you can withdraw the sample operating cradle at any time. If an error occurs during a test, you will hear a beep and a message displays on the screen.
11. Record the results on the Osmolality Patient Log and pull back the operating cradle to a positive stop.
12. Remove the sampler from the operating cradle. Depress the plunger in until the sampler tip is released and gently remove it from the end and place it into the biohazard waste bin. Gently wipe the white tip of the sampler with a lint free paper tissue to remove any residual sample. Place a new sample tip on the sampler.
13. Insert a clean clamber cleaner 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.
14. Repeat testing. If both results are within ± 2, report the original result. If results are not within ± 2, repeat a 3rd time. Use the two results that match and take the original result of the two matching results. If after repeating a 3rd time and results do not match, troubleshoot the analyzer. Review the operator's manual under the Troubleshooting & Service and Troubleshooting Table. DO NOT report patient results until troubleshooting resolves the issues and results are within ± 2.
15. Document results on Osmolality Patient/QC Log.
16. Enter results in LIS under worksheet ARCHE.

B. Procedure for 3320 model Osmometer:

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 (6mm) 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, non-ionic 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 level with the end of the tip or may be slightly concave.
5. Remove the chamber cleaner from the sample port and discard.
6. Scan Sunquest patient label before testing. The container identification number (CID) will appear on the instrument display and print-out after testing is complete.
7. Holding the sampler by the barrel, insert the tip into the sample port, then rest the sampler in the operating cradle.
8. To start the test, push the operating cradle in until it reaches a positive stop. It will take approximately one minute to perform testing. You can also start testing 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 the cradle. If the keypad was used, use the right "Cancel" key.
9. Record the results on the Osmolality Patient Log (attach print-out to the back of the log) and pull back the operating cradle to a positive stop.
10. Remove the sampler from the operating cradle. Depress the plunger in until the sampler tip is released and gently remove it from the end and place it into the non-biohazard waste bin. Gently wipe the white tip of the sampler with a lint free paper tissue to remove any residual sample. Place a new sample tip on the sampler.
11. Insert a clean chamber cleaner 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.
12. Repeat testing. If both results are within ±2, report the original result. If result are not within ±2, repeat a 3rd time. Use the two results that match and take the original result of the two matching results. If after repeating a 3rd time and results do not match, troubleshoot the analyzer, Review the operator's manual under the Troubleshooting & Service and Troubleshooting Table. DO NOT report patient results until troubleshooting resolve the issues and results are within ±2.
13. Document results on Osmolality Patient/QC Log. Also, attach patient print-out to the Osmolality/Patient QC Log.
14. Enter results in LIS under MCUA3.

STARTUP/SHUTDOWN: Turn the instrument on/off by pushing the rocker-style power switch on the instrument's back panel to the appropriate position. When turning on, there will be a short delay until the instrument's display becomes active.

SUPPLEMENTAL MATERIALS / ADDENDUM:
Osmolality Patient/QC Log
Osmometer Maintenance Log (MC)
Chemistry Daily Task Log (AR)

CALCULATIONS: N/A

INTERPRETING & REPORTING RESULTS:
Reference Ranges See Clinical Chemistry Information Sheet
Instructions for Computer Data Entry:

Result Entry
Select Manual Resulting Mode

- ARMC Enter "ARCHE" into worksheet text box
- Moses Cone Enter "MCUA3" into worksheet text box

Enter Specimen ID

Procedures for Results: All patient results should demonstrate repeatability. Precision (Repeatability) is demonstrated when the sample results are ±2 mOsm/kg H_2O of each other.

PROCEDURAL NOTES: N/A

RELATED PROCEDURES: N/A

LIMITATIONS OF PROCEDURE: If there is visible evidence of microbial growth in vials, do not use. Erroneous results can result from adverse shipping and/or storage conditions, use of expired materials or sample handling errors.

REFERENCES:
The Advanced® Osmo1 Single-Sample Micro-Osmometer User's Guide, Advanced Instruments Inc., Norwood, MA, Part No. 133005EN, Revision 3.
The Advanced® Micro-Osmometer Model 3320 User's Guide, Advanced Instruments Inc., Norwood, MA, Part No. 3325, Revision 11 010515.
Pesce, A.J.,Tietz, N.W., Methods in Clinical Chemistry, St. Louis, C.V. Mosby Co., 1987.
Bishop, M.L., Fody, E.P., Schoeff, L.E., Clinical Chemistry, 5th edition, Baltimore, Lippincott Williams & Wilkins, 2005.
Tietz, N.W., Fundamentals of Clinical Chemistry, 3rd Edition, Philadelphia, W.B. Saunders Co., 1987