Osmo1™ Single-Sample Micro-Osmometer
Clinical Lab Instruction

I. PURPOSE

This instruction describes how the Osmo1 Single-Sample Micro-Osmometer is used in clinical laboratories. This document provides instructions for clinical laboratories to perform osmolality testing on body fluids including plasma, serum, urine, and stool specimens. It includes instructions for processing, storing and testing specimens, and procedures for instrument calibration, quality control, and maintenance.

II. PRINCIPLE

Advanced Osmometers are devices for the determination of the concentration of solutions in terms of osmolality by means of freezing-point measurement. Advanced Osmometers utilize high precision thermistors to sense the sample temperature, to monitor the degree of supercooling and freeze induction, and to measure the freezing point of the sample. They can measure osmolality down to a resolution of 1 mOsm/kg H_2O.

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.
- Vapor pressure is lowered.

These are the so-called "colligative" or concentrative properties of the solution which, within reasonable limits, change in direct proportion to the solute concentration; in other words, 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.

Refer to the Osmo1 Single-Sample Micro-Osmometer User Guide for more detailed information on principles of freezing point osmometry.[1]

III. SAFETY

All specimens should be treated as potentially infectious and should be handled as if they are capable of transmitting disease. Precautions described in CDC and FDA recommendations and OSHA blood borne pathogen rules should be followed at all times when handling specimens and reagents.

IV. DEFINITIONS

Refer to the Osmo1 Single-Sample Micro-Osmometer User Guide for definitions.[1]

V. REQUIRED EQUIPMENT & MATERIALS

- Osmo1 Single-Sample Micro-Osmometer
- 20 µL Ease-Eject™ Sampler, Part # 3M0825
- Micro-Sample Test Kit, Part # 133800
- 50 mOsm/kg Calibration Standard, Part # 3MA005
- 850 mOsm/kg Calibration Standard, Part # 3MA085
- 2000 mOsm/kg Calibration Standard, Part # 3MA200 (optional)
- Clinitrol™ 290 Reference Solution, Part # 3MA029
- Osmolality Linearity Set, Part # 3LA028
- Protinol™ Protein-Based Controls, Part # 3MA028
- Renol™ Urine Osmolality Controls, Part # 3LA085
- Power cord
- Printer paper, Part # FLA835
- Lint-free, non-ionic paper
- User Guide (USB drive), Part # 133005UG

VI. QUALITY CONTROL

This section outlines the quality control materials and their intended use as recommended by Advanced Instruments for the Osmo1 Single-Sample Micro-Osmometer. It is recommended that laboratories incorporate these materials into their quality control management system. For quantitative tests, CAP requires that laboratories run 2 controls at 2 different concentrations daily or with each batch of samples/reagents.[2]

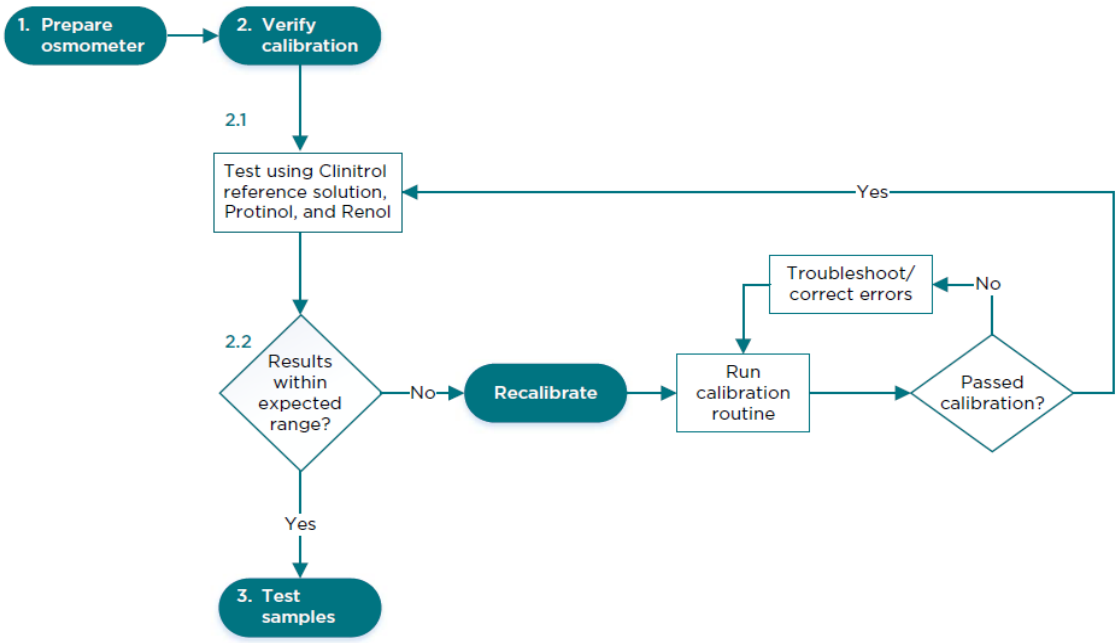
Prior to testing patient samples, osmometer operators should test **Clinitrol™ 290 Reference Solution** as part of your laboratory's quality control procedure. Clinitrol 290 Reference Solution is a NIST traceable reference designed to verify the osmometer's calibration. Clinitrol 290 single use vials should be discarded after a single day of use.

If you use your osmometer to test serum, plasma, stool or tissue homogenate you should test **Protinol™ Protein Based Serum Controls** as part of your quality control procedure prior to testing patient samples. Protinol Controls are formulated to mimic protein-based body fluids at 240, 280, and 320 mOsm/kg H_2O.

If you use your osmometer to test urine, you should test **Renol™ Urine Osmolality Controls** as part of your quality control procedure prior to testing patient samples. Renol Controls are formulated to mimic urine at 300 and 800 mOsm/kg H_2O.

NOTE: Use of third-party controls or calibrators will impact the instrument warranty and may affect instrument performance.

The following flowchart illustrates the recommended daily procedure.



Clinitrol™ 290 Reference Solution (Part # 3MA029)

- Clinitrol 290 Reference Solution is intended for use in evaluating the performance of your osmometer. It is a true reference designed specifically for osmometers and provides results that approximate the osmolality of normal serum. Use Clinitrol:
  - Daily, prior to testing samples, to verify instrument operation, and confirm calibration.
  - After performing a calibration.
  - To strengthen your laboratory's Quality Control program by using products made specifically for your osmometer.
  - Refer to the product insert for more detail

Protinol™ Protein-Based Osmolality Controls (Part # 3MA028)

- Protinol Protein-Based Controls are intended for use in evaluating the performance of your osmometer. Protinol Controls are formulated to mimic serum at 240, 280, and 320 mOsm/kg H_2O. Use Protinol:
  - Daily, prior to testing samples, to verify instrument operation, and confirm calibration.
  - After performing a calibration.
  - To strengthen your laboratory's Quality Control program by using products made specifically for your osmometer.
  - Refer to the product insert for more details.

Renol™ Urine Osmolality Controls (Part # 3LA085)

- Renol Urine Osmolality Controls are intended for use in evaluating the performance of your osmometer. Renol Controls are formulated to mimic urine at 300 and 800 mOsm/kg H_2O. Use Renol:
    - Daily, prior to testing samples, to verify instrument operation, and confirm calibration.
    - After performing a calibration.
    - To strengthen your laboratory's Quality Control program by using products made specifically for your osmometer.
    - Refer to the product insert for more details.

Osmolality Linearity Set (Part # 3LA028)

- The Osmolality Linearity Set is designed to help clinical laboratories easily monitor osmometer performance specifications and fulfill CLIA requirements for verifying the reportable range of a laboratory method. Use the linearity set:
    - To verify the linearity and reportable range of the instrument.
    - After performing a calibration.
    - Refer to the product insert for more details.

NOTE: Advanced Instruments does not recommend freezing the calibration standards, reference solutions, controls, or linearity sets.

Interpreting Results

Data generated using Advanced Instruments standards and reference solutions may be analyzed according to the accuracy and precision specifications of the instrument.

	Manufacturer's Specifications
Accuracy	• ±2 mOsm/kg H_2O from nominal value from 0 to 400 mOsm/kg H_2O (1 SD) • ±0.5% from nominal value from 400 to less than 1500 mOsm/kg H_2O (1 SD) • ±1% from nominal value from 1500 to 2000 mOsm/kg H_2O (1 SD)
Precision (Within-Run Repeatability)	• Standard deviation ≤2 mOsm/kg H_2O from 0 to 400 mOsm/kg H_2O • Coefficient of variation ≤0.5% from 400 to less than 1500 mOsm/kg H_2O • Coefficient of variation ≤1% from 1500 to 2000 mOsm/kg H_2O

Accuracy and precision (within-run repeatability) specifications apply to Advanced Instruments standards and reference solutions. Performance at Reference Conditions: 20°C to 25°C (68°F to 77°F); 40 to 60% relative humidity.

Expected Ranges

Refer to page 8 of the Osmo1 User Guide. Laboratories may choose to employ one, two, or three standard deviations for accuracy based on what is relevant in their laboratories. For normally distributed data, approximately 68% of the individual data values will fall within one standard deviation of the mean, approximately 95% within two standard deviations, and approximately 99.7% within three standard deviations. Expected ranges using two standard deviations are shown below for Clinitrol 290 Reference Solution and the Osmolality Linearity Set.

- Clinitrol 290 Reference Solution
  - 286-294 mOsm/kg H_2O (2 SD)
- Protinol Protein-Based Controls
  - Protinol 240 mOsm/kg H_2O: 233-247 mOsm/kg H_2O (per product insert)
  - Protinol 280 mOsm/kg H_2O: 273-287 mOsm/kg H_2O (per product insert)
  - Protinol 320 mOsm/kg H_2O: 313-327 mOsm/kg H_2O (per product insert)
- Renol Urine Osmolality Controls
  - Renol 300 mOsm/kg H_2O: 290-310 mOsm/kg H_2O (per product insert)
  - Renol 800 mOsm/kg H_2O: 790-810 mOsm/kg H_2O (per product insert)
- Osmolality Linearity Set
  - 100 mOsm/kg H_2O: 96-104 mOsm/kg H_2O (2 SD)
  - 500 mOsm/kg H_2O: 494-506 mOsm/kg H_2O (2 SD)
  - 900 mOsm/kg H_2O: 890-910 mOsm/kg H_2O (2 SD)
  - 1500 mOsm/kg H_2O: 1470-1530 mOsm/kg H_2O (2 SD)
  - 2000 mOsm/kg H_2O: 1960-2040 mOsm/kg H_2O (2 SD)

The Osmo1 includes several quality control tools. Control trends can be monitored using the on-board Levey-Jennings chart. The user may configure the expected value and limits for control solutions to fit his or her laboratory's current practices. Additionally, the user may access statistics (mean, standard deviation, and %CV) for up to 20 selected results.

VII. SAMPLE REQUIREMENTS

General

- 20 µL of sample is needed to perform each test.

Plasma & Serum

- Collection/Preparation
  - Follow your laboratory's procedure for collecting and preparing plasma and serum.
  - Evacuated tubes containing a gel for serum separation are acceptable for obtaining specimens for determination of osmolality.

- Storage
  - Plasma and 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 plasma and serum specimens is not possible, samples can be refrigerated or stored at room temperature for up to forty-eight (48) hours and can be tested for osmolality without significant bias. It is recommended, however, that users test serum specimens within twenty-four (24) hours of collection.[3]
  - Prior to analysis, plasma and serum specimens must be warmed to room temperature.

Urine

- Collection/preparation
  - Follow your laboratory's procedure for collecting and preparing urine.
  - Use containers without preservatives.
  - Centrifuge urine to remove gross particulate matter.

- Storage
  - Urine specimens should be tested as soon as possible after collection in order to obtain accurate osmolality results in clinical laboratories.
  - When immediate processing of urine specimens is not possible, they can be refrigerated or stored at room temperature for up to twenty-four (24) hours and can be tested for osmolality without significant bias.[4]
  - Prior to analysis, urine specimens must be warmed to room temperature.

Stool

- Collection/preparation
  - Follow your laboratory's procedure for collecting and preparing stool.

- Storage
  - Stool specimens should be tested as soon as possible after collection in order to obtain accurate osmolality results in clinical laboratories.
  - When immediate processing of stool specimens is not possible, they can be refrigerated to minimize the changes to the original osmolality due to evaporation or bacterial decomposition.
  - Prior to analysis, stool specimens must be warmed to room temperature.

VIII. CALIBRATION

Note: Refer to the User's Guide for detailed instructions.

Calibration procedure

1. From the Home screen, tap the menu icon. The main menu displays.
2. From the Main menu, tap **Calibration**. The system prompts you to log in.
3. Login and follow the on-screen instructions to test samples from each specified standard five times. You will test samples of known standards: either 50 and 850 mOsm/kg H_2O for a 2-point calibration; or 50, 850, or 2000 mOsm/kg H_2O for a 3-point calibration.
4. Upon completion of the last calibration test, the system displays a "Calibration successful" message or the reason for failure. Click OK to close the success (or failure) message.

IX. SAMPLE TEST PROCEDURE

Note: Refer to the User's Guide for detailed instructions.

1. *If applicable:* Log in with your User ID and enter the Sample ID.
2. Place a new sampling tip on the sampler with the plunger wire carefully inserted into the middle of the tip. Verify that the tip is straight and firmly seated.
3. With you thumb on the plunger top and fingers grasping the barrel, depress the plunger; then insert the tip into the liquid sample at least ¼" (6 mm) below the surface. Gently release the plunger to load a 20 µL sample.
4. Look at the sample you have just drawn. If there are voids or bubbles in the sample, discard it and load another sample that does not contain voids.
5. Remove any sample on the outside of the tip using a clean, lint-free, non-ionic paper. Quickly swipe the end of the sampler tip to remove any excess sample protruding beyond the tip. Be careful not to remove any of the sample from inside the tip.
6. Holding the sampler by the barrel, carefully insert the tip into the sample port; then rest the sampler body in the operating cradle.
7. Grasp the operating cradle and push it slowly forward until you feel a positive stop. The test starts when the cradle reaches the forward position.
8. Wait while the Osmo1 performs the tests. When the test completes, the resulting osmolality displays in the middle of the screen.
9. Withdraw the operating cradle and remove the sampler from the cradle.
10. Grasp the sampler tip and depress the plunger to help remove it. Discard the sampler tip.
11. Wipe the plunger tip with a clean, lint-free, non-ionic paper, being careful not to dislodge the Teflon tip.
12. Insert a clean, dry clamber cleaner into the sample port until you feel a positive stop. Rotate four or five times in one direction while applying forward pressure.
13. Withdraw the cleaner and use the other end to clean the probe again in the same manner. Leave the cleaner in the sample port until the next test.
14. To test additional samples, repeat this procedure.

X. MAINTENANCE

Note: Refer to the User's Guide for detailed instructions.

Osmometer

1. Cleaning the instrument exterior – Periodically wipe the instrument with a barely-damp cloth. User either warm soapy water or isopropyl alcohol to dampen the cloth.
2. Cleaning air vents – Make sure the fan on the back of the instrument has no accumulated dust and debris that could impede air flow. Dirty air vents can cause instrument overheating and reboots.
3. 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.
4. Cleaning the Solenoid – A dirty solenoid can cause "Sample Did Not Freeze" errors and can affect instrument accuracy and repeatability. Instruments used daily should be cleaned monthly, while instruments that are used less frequently should be cleaned every three months.
5. Replacing the fuse – If you determine that your instrument is not functioning because of blown fuses, you will need to replace the fuses.

20 µL Ease-Eject™ Sampler

To ensure proper instrument operation: you should replace the plunger wire tip of the sampler every 500 tests (or every time you empty a Micro-Sample Test Kit). Failure to replace the plunger wire may affect instrument accuracy and repeatability.

Note: A sampler plunger wire is included with each Micro-Sample Test Kit.

XI. REFERENCE RANGES[5]

Normal Serum Reference Ranges

- Neonate: May be as low as 266 mOsm/kg H_2O.
- Child/Adult: 275-295 mOsm/kg H_2O.
- >60 Years: 280-301 mOsm/kg H_2O.

Normal Urine Reference Ranges

- Random: 50-1200 mOsm/kg H_2O, depending on fluid intake.
- Random: >850 mOsm/kg H_2O, after 12 hour fluid restriction.
- 24 Hour: ~ 300-900 mOsm/kg H_2O.

Normal Stool Reference Ranges

- Normal fecal fluid has an osmolality similar to that of serum.

XII. LIMITATIONS

Note: Refer to the User's Guide.

In a simple solution (i.e., 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. The concentration of each solute cannot be easily determined.

Interfering Substances

- The data below was collected following CLSI document EP7 *Interference Testing In Clinical Chemistry*.[6]

  No interference was observed on the following interferents up to and including these concentrations[7]:

Hemolyzed Serum	Icteric Serum	Lipemic Serum
31.25 mg/dL hemoglobin	25.1 mg/dL bilirubin	1557 mg/dL triglycerides

Reportable Range (Analytical Measurement Range)

- 0 to 2000 mOsm/kg H_2O

XIII. REFERENCES

1. Osmo1 Single-Sample Micro-Osmometer User Guide. Norwood, MA. Advanced Instruments.

2. CAP Chemistry Checklist revised 7/28/2015, CHM.13900 Daily QC - Nonwaived Tests.

3. Curria A, et. al. 2009. Refrigerated and Room Temperature Storage Stability of Serum Osmolality Measurements. Advanced Instruments Technical Literature.

4. Curria A, 2010. Refrigerated and Room Temperature Storage Stability of Urine Osmolality Measurements. Advanced Instruments Technical Literature.

5. Burtis, C.A., Ashwood, E.R., 1999. Tietz Textbook of Clinical Chemistry, Third Edition.

6. NCCLS. *Interference Testing in Clinical Chemistry; Approved Guideline.* NCCLS document EP7-A (ISBN 1-56238-480-5). CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA 2002.

7. Rosenman J, et. al. 2010. Effects of Hemolysis, Icterus, and Lipemia on Serum Osmolality Results using the Advanced® Model 3250 Single-Sample Osmometer. Advanced Instruments Technical Literature.