Laboratory Procedure Manual

Analyte: **Osmolality**

Matrix: **Urine**

Method: Freezing point reduction
Osmette II, Model 5005
Automatic Osmometer

Method No.:

Revised: December 2010

as performed by: *Mobile Examination Center (NHANES)*

Contact: *Brenda Lewis, MS*

December 2010

Important Information for Users

NHANES periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set Information

This document details the Lab Protocol for NHANES 2009–2010 data.

A tabular list of the released analytes follows:

File Name	Variable Name	SAS Label (and SI units)
UROSMO_F	URXOAV	Urine Osmolality (mOsm/kg)

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

A. Test Principle

The urine osmolality determination is based upon the principle that increased concentration of a solute in a solution causes lowering of its freezing point. This method is referred to as freezing point depression osmometry. A sample of the specimen to be analyzed is aspirated into the sample tube, which is then placed in the cooling chamber of the osmometer. The sample is super cooled below the freezing point. Then crystallization is initiated by rapidly vibrating the sample to seed it with air bubbles. After seeding, the sample temperature rises because of the heat of fusion released during the freezing process. The temperature rises until the equilibrium plateau is reached (Figure 12-1). During the equilibrium plateau only a small fraction of the water is frozen. The sample continues to freeze as the temperature begins to decrease again because of the colder environment.

Figure 12-1. Typical cooling curve

Crystallization causes the release of the heat of fusion of water causing a constant temperature or a plateau. This temperature plateau is the freezing point of the urine specimen and is measured by the use of thermistors. Osmometers read directly in mOsm/kg (milli osmoles per kilogram of water) water by converting the thermistor probe (a precise temperature sensor) readings and directly comparing it with readings obtained using standard aqueous salt solutions of known osmolality (Standards).

The temperature at the plateau is the freezing point of the sample and can be converted to units of osmolality (osmotic concentration) by observing that 1.0 osmole depresses the freezing point of water by 1.858^{o}C, where 1.0 osmole = 1.0 mole of osmotically active particles.

The most commonly used technique for converting the reading to units of mOsm/kg H_2O is direct comparison with Standards of known osmolality. Osmolality is expressed in units of milliOsmoles (mOsm) per kg of water or mOsm/kg where one mOsm is equivalent to one millimole of dissolved solute particles. A solution containing 1 osmole (1000 mOsm) of dissolved solute per kg of water lowers the freezing point of water by 1.858^{o}C. Thus, the freezing point depression of the urine specimen can be converted to units of osmolality or osmotic concentration by dividing it by 1.858.

B. Clinical Relevance

The osmolality test is a determination of the number of osmotically active solutes present in the urine. It is performed to help evaluate the body's water balance and its ability to produce and concentrate urine. Increased urine output may be due to increased fluid intake, lack of appropriate amounts of ADH, or diabetes mellitus (increased glucose levels leading to increased urine output). Decreased urine output may be due to a variety of causes including decreased blood flow to the kidneys, an appropriate response to dehydration, or damage to tubular cells in the kidneys. Urine sodium and creatinine are often performed along with urine osmolality.

Sometimes a urine osmotic gap is calculated and used to help evaluate the kidney's ability to excrete acid and reabsorb bicarbonate, to detect the presence of osmotically active molecules, and to compare with the plasma osmotic gap.

Urine osmolality is dynamic and will fluctuate as the body responds to and corrects temporary water imbalances. Urine osmolality tests must be evaluated in the context of the participant's clinical presentation and along with the findings of other tests, such as sodium, glucose, and BUN. When participants have increased urine output and a low osmolality, then they are either ridding their body of excess fluids or unable to concentrate urine appropriately (which may be due to diabetes insipidus, a lack of ADH). If they have increased urine output and a high osmolality, then it may be due to diabetes mellitus. If participants have decreased urine output and high osmolality, they may be dehydrated; if they have low or normal osmolality, they may have kidney damage. Osmolality results are not diagnostic; they suggest that a participant has an imbalance but they do not pinpoint the cause.

Urine osmolality may be increased with:

- congestive heart failure
- hypernatremia
- inappropriate ADH secretion
- liver damage
- shock

Urine osmolality may be decreased with:

- diabetes insipidus
- excess fluid intake
- hypercalcemia
- hypokalemia
- kidney tubular damage

Within NHANES the osmolality test will be used in conjunction with the urine flow rate. The urine flow rate evaluates the weight of solids in water. Osmolality and urine flow rate usually change in parallel to each other. When large and heavy molecules (such as glucose and protein) are present in the urine, however, the results will diverge. The flow rate will be increased more, due to the weight of the molecules, while urine osmolality will be increased less, reflecting the number of molecules.

2. SAFETY PRECAUTIONS

All specimens may be potentially positive for infectious agents including HIV and the hepatitis B and C viruses. Observe universal precautions. It is mandatory to wear gloves and a lab coat when handling all human urine products and Quantimetrix® controls. Dispose of all biological samples in a biohazard container and wipe down all work surfaces with 10 percent bleach solution at the end of each session.

The mobile examination center (MEC) *Working Safely with Hazardous Chemicals* manual contains the material safety data sheets (MSDS) for both the Precision Systems Standards and CON-TROL solutions and the Quantimetrix Human Urine Controls.

Neither the Precision Systems Standards nor CON-TROL solutions, nor the Quantimetrix Human Urine controls contain any ingredients that have been determined to be health hazards pursuant to Federal OSHA standards.

The Quantimetrix Human Urine Controls contains human urine. It is recommended that such samples be handled at the Centers for Disease Control Biosafety Level 2.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

A. Integrated Survey and Information System (ISIS)

The Osmette II automatically transmits successfully completed SP results to the MEC automated ISIS system. The session number and technologist ID are automatically captured. The medical technologist reviews all SP results in the Osmolality module within the ISIS Laboratory application. The Osmolality QC module captures the QC results from the osmometer. The medical technologist enters the control lot number and expiration date. The Laboratory application evaluates the osmolality data for accuracy based on a preset precision limit. The final decision to accept or reject a result is the responsibility of the medical technologist.

All data are backed up and stored at Westat's home office.

4. SPECIMEN COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

A. Specimen Collection

The urine sample is collected as a clean catch in a sterile 500-mL capacity container. The volume of the collection is calculated and the medical technologist records the sample as collected in the ISIS Laboratory application.

B. Specimen Preparation

The technologist prepares for the urine osmolality test by recording urine collection as either complete or quantity not sufficient (QNS). Once at least 2-mL of urine has been collected on female SPs eligible for pregnancy tests, or 1-mL on all other SPs eligible for urine collection, the urine osmolality test may be completed on the SP. The urine osmolality test requires at least 1-mL of urine. Do not perform osmolality on urines that are cloudy or bloody. Do not spin urine prior to performing the osmolality.

QNS urine samples should be held at ambient temperature until enough urine is obtained from the SP and pooled with the previous specimen(s). This pooling process results in the urine having the same concentration as the environmental samples.

5. PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

The ISIS Laboratory application stores and maintains the lot numbers and expiration dates of QC controls and calibration standards.

A. Equipment

1. OSMETTE II™ Model 5005, Automatic Osmometer (Precision Systems, Inc). The four Osmette II serial numbers and locations are:

Osmette	Location	Serial Number	NCHS Property Tag
1	MEC 1	LL0809C	174145
2	MEC 2	LL0807C	17412
3	MEC 3	LL0808C	174144
4	Westat (backup)	LL0806C	174113

2. 10 µl Pipette (Precision Systems, Inc.). Use one pipette in each MEC. Keep one backup pipette in each MEC and two backup pipettes in the NHANES warehouse.

B. Calibrators

Use the Precision Systems Osmometry Standards to calibrate the Osmette II machines and validate the calibration. Use the Precision Systems CON-TROL reference standard glass ampules to validate the Standards. Both are manufactured by Precision Systems:

Precision Systems, Inc.
16 Tech Circle

Natick, MA 01760-1029
508-655-7010

1. Precision Systems Osmometry Standards are aqueous solutions of known osmolality containing high purity sodium chloride for the calibration of osmometers. Standard osmolalities are listed on the bottle label. Acceptable ranges are used for quality control only.

   - 100 mOsm, 125-mL bottle (catalogue #2101) – acceptable range 96-104 mOsm
   - 500 mOsm, 125-mL bottle (catalogue #2105) – acceptable range 496-504 mOsm
   - 1,500 mOsm, 125-mL bottle (catalogue #2115) – acceptable range 1485-1515 mOsm
   - 2,000 mOsm, 125-mL bottle (catalogue #2120) – acceptable range 1980-2020 mOsm

2. Precision Systems CON-TROL reference standards are aqueous solutions of known osmolality containing high purity sodium chloride for validation of calibration standards. CON-TROL osmolalities are listed on the glass ampule.

   - CON-TROL 100, 100 mOsm reference solution, 5mL ampule (catalogue #2201) – acceptable range 98-102 mOsm
   - CON-TROL 500, 500 mOsm reference solution, 5mL ampule (catalogue #2203) – acceptable range 496-504 mOsm

C. Controls

The Quantimetrix Human Urine Controls are intended as a means of monitoring human urine assay methods to validate quantification of patient samples. Control materials having known component concentrations are an integral part of diagnostic procedures. Daily monitoring of control values establishes intralaboratory parameters for accuracy and precision of the test method. The controls are manufactured by the Quantimetrix Corporation.

Quantimetrix
2005 Manhattan Beach Blvd.
Redondo Beach, CA 90278-1205
(310) 563-0006

1. Quantimetrix Human Urine Control, Level 1 and 2 are supplied as a ready-to-use liquid requiring no reconstitution. They are prepared from human urine. The Human Urine Controls are fortified to target levels with human proteins and reagent grade chemicals. Preservatives have been added to inhibit microbial growth.

   - Human Urine Control, Level 1 Catalog #1431-31, three 10-mL bottles
   - Human Urine Control, Level 2 Catalog #1432-31, three 10-mL bottles

D. Supplies

1. 50-ml Precision Systems sample tubes (Precision Systems catalog #2023)

2. Teflon pipette tip piston replacements (Precision Systems catalog #2036)
3. 10 µl disposable pipette tips (Precision Systems catalog #2048)
4. Cleanettes (Precision Systems catalog #2048)
5. Distilled water
6. Kimwipes
7. Latex or nitrile gloves
8. Disposable lab jacket – 48 inches long
9. Dow 311 Valve Lubricant

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

The Calibration procedure is recommended for reporting sample values from 0 to 2000 mOsm.

Calibration is a procedure to standardize the instrument by determining its deviation from calibration references and to apply any necessary correction factors. The Osmette II stores calibrations. All subsequent valid calibration values are added and averaged into previous calibrations.

A. Precalibration

Perform a calibration after the instrument has been turned on and running for at least 10 to 15 minutes to allow for the Osmette temperature to completely equilibriate.

1. Precalibration procedure
    a. Run a 100 mOsm standard through the machine to clean the well and ensure that the electronics and cooler are cycling correctly.

B. Osmette Total Calibration with Standards

Use the Precision Systems 100, 500, 1,500, and 2,000 standards to complete a total calibration. Perform a total calibration:

- At the start of each stand before analyzing samples.
- After cleaning or doing any maintenance on the equipment.
- If the error message “OUTSIDE CALIBRATION” displays.
- If the Precision Systems Representative suggests a calibration.
- Acceptable ranges for Standards used in the calibration procedure:
    - Water and 100 Standard – acceptable range +/-1
    - 500 Standard – acceptable range +/-2

- 1500 Standard – acceptable range +/-4
- 2000 Standard – acceptable range +/-6

- Note: Run each standard three times. Each standard can be run up to six times as the instrument uses each standard result as a running average. If the standards are out of range, the calibration must be started over by exiting the calibration mode and then re-entering the calibration mode.

C. Calibration Procedure

1. Press "CALIBRATE" button.
2. Wipe the well with a Cleanette.
3. Run two distilled water samples. Record the result reading of each in the Lab Start of Stand QC Module.
4. Run two 100 mOsm Standards. Record the result reading of each in the Lab Start of Stand QC Module. Wipe the sample well with a Cleanette.
5. Run three 500 mOsm Standards. Record the result reading of each in the Lab Start of Stand QC Module. Wipe the sample well with a Cleanette.
6. Run three 1,500 mOsm Standards. Record the result reading of each in the Lab Start of Stand QC Module. Wipe the sample well with a Cleanette.
7. Run three 2,000 mOsm Standards. Record the result reading of each in the Lab Start of Stand QC Module. Wipe the sample well with a Cleanette.
8. Press the "CALIBRATE" button again to complete the calibration process. If the "CALIBRATE" button is not pressed again at the end of calibration, the calibration will not be saved.

D. Osmette Troubleshooting Calibration with Standards When QC is Not Within the Acceptable Range

- Note: Run each standard three times. Each standard can be run up to six times as the instrument uses each standard result as a running average. If the standards are out of range, the calibration must be started over by exiting the calibration mode and then re-entering the calibration mode.

- Acceptable ranges for Standards used in the calibration procedure:
- Water and 100 Standard – acceptable range +/-1
- 500 Standards – acceptable range +/-2
- 1500 Standard – acceptable range +/-4

- 2000 Standard – acceptable range +/-6

1. Run a 100 mOsm standard through the machine to clean the well and ensure the electronics and cooler are cycling correctly.

2. Press “CAL” button.

3. Run two100 mOsm reference standards. Wipe the sample well with a Cleanette.

4. Run three 500 mOsm reference standards. Wipe the sample well with a Cleanette.

5. Run three 1500 mOsm Standards. Record the result reading of each in the Lab Start of Stand QC Module. Wipe the sample well with a Cleanette.

6. Run three 2000 mOsm Standards. Record the result reading of each in the Lab Start of Stand QC Module. Wipe the sample well with a Cleanette.

7. Press the “CALIBRATE” button again to complete the calibration process. If you do not press the “CALIBRATE” button again at the end of calibration, the calibration will not be saved.

8. PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

Run SP urine collection samples in duplicate and average the two results. If the difference between the two values exceeds 40 (four times the SD of 10%), then run the sample a third time. Average the closest two of the three results. If the final result is based on a single run, then a comment “results based on single run” will automatically be appended to the result. Results are saved in whole numbers

Osmolality test results will not be reported back to the participant. There are no panic or action limits for this test. If the measured value is >2,000 mOsm/kg, then the result will be reported as “2,000” with “out-of-range” comment.

A. Osmette II Instrument Placement

Allow at least 3 inches on both sides and back of the instrument for placement of the Osmette. The Osmette should not be placed near a heat source or in direct sunlight. Do not block the air outlet (at rear of instrument) and the air intake (at front right side of instrument). Any blockage of the air

intake or outlet will slow the cooling of the Cooler Well, so that it will take longer to reach operating temperature. The instrument work area must be free of vibration (avoid benches with centrifuges).

B. Instrument Buttons and Functions

1. Plug the OSMETTE II into a grounded AC outlet of the proper voltage (115 v AC).

2. The ON/OFF button is located on the rear of the machine. It is a black rocker button. Always make sure the button is in the OFF position when plugging in the instrument.

3. The SPEC button is an unlabeled button on the rear of the machine. The button is a thin white unlabeled stick projecting from the back of the instrument. This button frequently acts as a cancel button. Stops both stabilizing, reading, digit entry, or any special function: acts as an escape key.

4. The CALIBRATE button initiates instrument calibration. The CAL button is located on the right front console of the instrument.

5. The RUN button initiates running a reading, whether calibrating or running an unknown. The RUN button is located on the right front console of the instrument.

6. The shutter is a metal cover that will automatically close and protect the cooler well. The shutter opens when the instrument is turned on. It will close to clamp around the pipette when a sample is run. It will close after approximately 10 minutes of inactivity to protect the cooler well from dust and debris. Always check to ensure the shutter is open before wiping the cooler well or running a sample. To open the shutter after it has automatically closed, the instrument operator must manually open the shutter:

   - Press the SPEC button on the back of the machine.
   - Press the run key twice to display “SPEC2” on the Osmette screen.
   - Press the CALIBRATE key. The shutter will open.

   The shutter closes after 10 minutes of inactivity regardless of the pipette or Cleanette sitting in the cooler well. The medical technologist should avoid leaving the pipette in the instrument after a sample has been run. The medical technologist should also not leave Cleanettes in the cooler well between samples. If a sample (or Cleanette) is left sitting in the cooler well and the shutter has clamped around it, follow the above instructions to open the shutter. Do not manually force it open or pull the pipette or Cleanette out.

C. Proper Pipetting Technique

Proper pipetting technique is critical to accurate osmolality results. Take several steps to ensure that the pipette is functioning properly:

1. Check the pipette calibration daily as described in VIII. F and Section 15.10.

2. Wipe the pipette wire and Teflon tip with a Kimwipe before attaching a disposable pipette tip. This will absorb any residual moisture from the previous sample and prevent

inadvertently drawing the sample too far up into the pipette tip from residual liquid attraction.

3. When placing a disposable pipette tip on the pipette, ensure that the pipette tip snaps onto the pipette. If it is not properly seated, the entire pipette tip will come off when the piston is depressed. Check this by depressing the pipette piston once when a new pipette tip is attached and before drawing the sample. If the pipette tip immediately comes off, firmly reattach it. If that does not correct the problem, discard the pipette tip and affix another one to the pipette. Recheck.

4. If the disposable pipette tip is properly seated on the pipette and the Teflon tip does not give any resistance against the pipette tip when depressing the pipette piston, change the Teflon tip as described in Section 15.10.

5. Ensure that, when the pipette is placed in the sample, that the pipette tip is fully submerged to avoid accidentally drawing air bubbles in the sample.

6. Wipe the end of the pipette tip with a Kimwipe after drawing the sample. Make sure that the end of the drawn sample is flush with the very tip of the pipette. Over wiping the sample may draw some of the sample out of the end of the pipette and lead to premature sample freezing from air bubbles. If the sample is not flush with the end of the pipette, discard the sample and pipette tip and redraw the sample.

7. Check to make sure there are no other air bubbles in the sample. If there are other air bubbles visible in the sample, discard the sample and pipette tip and redraw the sample.

8. Always check the air bubble between the sample and the Teflon tip. Familiarity with the correct air bubble size is critical to recognizing a properly functioning pipette. The correct air bubble size is shown in Exhibit 12-1. If the air bubble appears smaller or larger than the picture, dispose of the plastic pipette tip and drawn sample and check the pipette calibration. Check to make sure sufficient resistance is felt from the Teflon tip when a new disposable pipette tip is inserted on the instrument. Inspect the pipette for any undetected damage, such as a bent pipette wire. Ensure that there is enough sample for a full sample draw and that the disposable pipette tip is properly seated on the instrument.

D. Running SP Samples

It is **critical** that the Osmo application is running on the ISIS computer before running any SP samples. This application captures results from the Osmette II. Any samples run without the Osmo application running will be lost and will need to be rerun. To open the Osmo application, double click on the icon of a pipette on the desktop:

The application icon will display in the system tray when the application is running. It is very faint, but will turn green when data are transmitted from the Osmette to the ISIS computer. Double check to make sure this icon is visible before running SP samples.

1. Switch (at rear of instrument) to ON, and allow the cooler well to cool down. Allow the instrument to run for 15 minutes to warm up to equilibrate all the circuitry before running SP samples or QC.

2. Notice that the green display turns on, displaying "COOLER STABILIZING."

3. In a few minutes, the display will change to "WIPE BEFORE NEXT SAMPLE," indicating that the refrigeration system has reached operation temperature and the instrument is ready to use. Do not attempt to use the OSMETTE until this change, as the refrigeration system will be too warm to give satisfactory results.

4. Push a Cleanette all the way to the bottom of the cooler well. The Cleanette is placed in the cooler well and twisted to soak up moisture from the previous samples. The tip of the Cleanette will cover the probe when inserted all the way into the well. Rotate it to remove any moisture. Be careful to insert the Cleanette in a straight down motion. Twist the Cleanette in the cooler well by spinning the Cleanette between thumb and forefingers – do not bend the Cleanette while wiping the well. Remove it by pulling it straight out to avoid bending the probe. Remove and dispose of the Cleanette after use. A fresh Cleanette is used with each sample.

   Do not place a sample in an unwiped cooler well.

5. Examine the urine collection sample. If blood is present, do not run the sample. This is to protect the probe from additional protein. Mark the sample as not done with comment code "blood present." If particulate matter is present, centrifuge the sample before running as described in Section IV.B.

6. Attach a clean, dry pipette tip to the pipette. Verify the function of the pipette as described in Section VII.B.

7. Scan the bar code (Figure 12-2) on the urine collection cup.

8. Using the Osmette II pipette draw 10 uL of sample directly out of the urine collection cup. Carefully pipette samples to avoid capturing air in the bottom of the pipette tip. Dry the outside of the pipette tip with a Kimwipe tissue. Do not wipe the pipette with any other material.

9. Place the pipette tip in the cooler well. The Thermistor Probe is located at the bottom center of the cooler well. The pipette places the pipette tip with the sample into the bottom of the cooler well, surrounding the Thermistor Probe with the sample. The sample remains in the pipette tip, with the pipette attached, when it is placed in the cooler well.

10. Press RUN to initiate the osmolality test. The instrument screen will display the sample ID of the specimen. If no sample ID is displayed, use the SPEC button to cancel the test. Scan in the bar code for the sample before rerunning the test.

11. Observe the Display: The number displayed is an indication of the approach to the seeding temperature. The sample is fully cooled when this number decreases to 0. During this process the Osmette shutter will clamp onto the pipette to secure the pipette for seeding.

    Do not attempt to remove the pipette while the shutter is clamped to the pipette.

12. When the display reaches approximately 0, the sample is automatically seeded by a vibration of the shutter. If the display does not reach 0, and vibration does not occur, READ will not be displayed, and no reading can be made. Rerun the sample.

13. After a few seconds, READ will be displayed, together with the result in mOsm. The shutter will then open to allow for removal of the pipette and for cleaning the cooler well.

14. The osmolality measurement is complete. Before leaving the instrument or placing a new sample in the cooler well, use a fresh Cleanette to wipe the cooler well.

    Do not leave the Cleanette in the cooler well.

15. Eject the used pipette tip into the biohazards container and wipe the Teflon tip of the pipette with a Kimwipe before attaching a new disposable pipette tip for the next sample.

E. Daily Shut-down Procedures

1. Wipe cooler well with a Cleanette before turning off the instrument. This is to ensure that there is no residual moisture in the cooler well before shutting off the instrument.

2. Turn off the instrument using the rocker button located on the back of the instrument. Turn the instrument off after each session. There is no standby mode for the instrument and turning it off when not in use will prolong the instrument's life.

F. Verifying the Pipette Calibration

The pipette requires two small instruments to verify that it is calibrated correctly and will draw the proper amount of solution into the disposable pipette tip. One calibrator resides in the clear red box with the replacement Teflon tips. This calibrator is made of a hard black plastic and is about an inch long. It has a nib on one end. This calibrator measures the size of the bubble that is held between the Teflon tip and the sample when in the disposable pipette tip. To ensure the bubble is the proper size place a clean disposable pipette tip on the pipette. Depress the plunger. Insert the nib into the end of the disposable pipette tip. The end of the nib should just touch the end of the Teflon tip; the tip should not push the tool out, and oppositely there should be no space gap between the tool and the Teflon tip.

If the bubble is not properly calibrated, remove the disposable pipette tip and unscrew the lock nut at the base of the pipette wire. Hold the plunger firmly with one hand while unscrewing the lock nut with the other. Use a pair of pliers if necessary. Unscrew the lock nut until it is loose. The pipette wire will also feel loose. Be careful not to turn the pipette wire accidentally when unscrewing the lock nut. Controlled, small turns will adjust the pipette wire to change the calibration setting. Turn the pipette wire in a clockwise direction (if looking at the end of the pipette tip head on) to pull the pipette tip up

into the pipette and therefore make the bubble size larger. Turn the wire clockwise to adjust the pipette so the calibration tool will insert further up into the end of the clean pipette tip. Turning the wire counterclockwise adjusts the pipette so that the calibration tool will close the gap between the calibration tool and the Teflon tip. The tool and the tip should just touch when properly calibrated. When the desired adjustment has been made, tighten the lock nut down with your fingers.

Place a clean disposable pipette tip on the pipette. Use the black calibration tool again to check the bubble size. Readjust if needed by following the above procedure. Once the calibration is successful, tighten the lock nut so that it will not come loose during regular use. Be careful not to damage the pipette.

The second instrument required to calibrate the pipette resides on the pipette itself. The end of the plunger opposite the tip has a tool that reads "Calib Gage & Key." This tool can be unscrewed from the end. The length of the tool measures the correct draw amount for the pipette. To measure the draw amount place a clean disposable pipette tip on the pipette. Without depressing the plunger, insert the calibration gage into the end of the disposable pipette tip. The end of the calibration gage should just touch the end of the Teflon tip.

If the draw amount is not properly calibrated, twist the pipette plunger around until the nut on the inside of the plunger aligns with the hole in the side of the pipette body. Use the tiny hex wrench on the end of the calibration gauge to carefully turn – usually not more than a quarter turn – the nut inside the hole to loosen plunger. Use the slack in plunger to move wire with Teflon tip towards end of pipette tip. Use the hex wrench as a guage to push the wire with Teflon tip back so it's calibrated. Immediately tighten the hex nut. Verify that the pipette is calibrated by depressing the piston and use the calibration gage to see if the draw amount is now correct. Readjust if needed by following the above procedure. Be sure to replace the calibration gage in the end of the pipette when calibration is complete.

Changing the Osmette II Pipette Teflon Tip

Change the Teflon tip if any of the below are encountered:

- The beginning of each stand.
- The resistance of the tip when depressing the pipette appears to be too loose.
- The air bubble separation above the sample is gone after a reading.
- The readings are erratic.

Replace the Teflon pipette tip. Remove the pipette tip from the disposable pipette tip or the protective cover. The Teflon tip is the tiny white plastic on the end of the pipette wire. The tip can be removed several ways. Any method is acceptable as long as care is taken to not bend the pipette wire or damage the pipette in any other way. A razor blade can be used to carefully cut along the side of the tip to cause it to release from the end of the wire. The tip will also come off by using a fingernail and some patience to work a space between the tip and wire and then push the tip off.

Locate the small clear red box labeled "Osmette Teflon Tips and Calibrator." Use one of the new Teflon tips inside the box as the replacement. The Teflon tip has one end that is solid and one end with a hole. The end with the hole should be placed on the end of the pipette wire. Ensure that the wire is fully inserted into the new tip. There should be no gap between the white tip and the pipette wire.

G. Cleaning, Opening, and Oiling the Osmette II Probe

The Osmette II may need to have the probe cleaned for several reasons:

- The instrument displays a large amount of "NO FREEZE" errors.
- The instrument displays a large amount of "PRE FREEZE" errors.
- The readings are erratic.

Clean the Osmette II probe. Wet the end of a Cleanette with and alcohol prep pad. Use as many pads as needed to ensure the end of the Cleanette is wet. Insert the wet end of the Cleanette into the probe well and wipe by rotating the Cleanette left and right. Remove the Cleanette. Wet the end of a Cleanette with distilled water. Insert the wet end of the Cleanette into the probe well and wipe by rotating the Cleanette left and right. Remove the Cleanette. Insert a dry Cleanette into the probe well and wipe by rotating the Cleanette left and right. Remove the Cleanette. This process should clean any residual proteins left in the well.

Over time some sample may leak out into the probe chamber because of leakage due to loss of surface tension (as in the case of proteins in the sample or improperly drawn samples). The only way to clean this residue is to open the probe chamber and wipe off the entire probe, base, and chamber. Open the Osmette II for cleaning the probe chamber. Ensure that the power button on the back of the Osmette is switched to "Off." Unplug the instrument from both the electrical outlet and the computer. Very gently turn the instrument over and lay it on its top so that the underside is tilted towards you. The bottom of the instrument is exposed. There is a black plastic piece on the underside of the instrument known as the "coffin" for its shape. The coffin acts as a cover for the probe. Open the coffin by using a Phillips head screwdriver to loosen the single screw on the left hand, broad end of the coffin. Carefully remove the loose screw and place it somewhere secure so it will not get lost. The loose end of the coffin can now swing up to reveal an opening underneath.

Inside the opening is a large black piece with a white circle in the middle. The white circle is made out of a rubbery, softer material. In the white circle is a white hard plastic donut with a white wire coming out. Using gloved hands, use your fingertips to gently unscrew the white donut by turning it to the left. Once the donut is loose, gently pull it straight out of the probe chamber, being careful not to touch the probe tip to the sides of the chamber. The probe is on the end of the white plastic donut. It is made of a fine metal wire, much like a pin. At its base is a flat surface with a black circle surrounded by a metal circle.

To clean the Osmette II, use only a Cleanette to wipe around the base of the probe (black circle and metal circle) to remove any residual sample not picked up by normal cleaning. Look down into the probe chamber on the instrument. On each side of the chamber are threads to fasten the donut back in place. Use a cleanette to wipe around the threads and the ring at the bottom of the chamber to absorb any residual sample.

On a rare occasion the probe base may need to be oiled. Only oil the probe if recommended by the Precision Systems technician. To oil the probe use a tiny dab of Dow 311 industrial lubricant on the end of a Cleanette. Wipe the lubricant around the base of the probe (black and metal circles). The lubricant should be spread very thinly. The purpose of the lubricant is to ensure that no sample seeps down into the break between the black circle and the metal circle at the base of the probe. The lubricant also helps to create surface tension to hold the sample in the pipette tip. Use a clean Cleanette to wipe off any globs of lubricant on the base of the probe.

Once cleaning and/or oiling is complete, put the Osmette II back together. Carefully direct the probe back down into the probe chamber. Use your fingers to gently tighten the white plastic donut into the white circle. The donut should only be finger tight; do not overtighten. Swing the coffin back down into place so that the hole in the coffin for the screw lines up with the hole in the osmette, and the probe opening is completely covered. Use the screw and Phillips head screwdriver to fasten in place. Do not over fasten.

9. REPORTABLE RANGE OF RESULTS

The Osmette II is intended to measure aqueous solution concentrations from 0-200 mOsm/kg H_2O.

10. QUALITY CONTROL (QC) PROCEDURES

A. Standards and CON-TROL Principle

Both the Precision Systems Standards and CON-TROL Standards are aqueous solutions of known osmolality. CON-TROL Standards are used to verify the accuracy of the Standards by equal comparison. The Standards verify the accuracy of the Osmette II instrument by running a solution of known osmolality as an unknown.

B. Standards and CON-TROL Storage and Handling

Precision Systems Standards should be stored in upright bottles at room temperature. They should not be diluted.

Always:

- Squeeze a small amount of the Standards solution into a disposable container or into the sink. This is to clear out the pour spout and remove any salt crystals that may have formed.
- Pour (never pipette) the Standards into the Precision Systems sample tubes and immediately snap the cap shut on the sample tubes. Clearly label the tubes with the Standards level using a permanent marker.
- Use only the amount of solution needed to gain acceptable pipetting technique and results. Under normal circumstances, this will be about two to five drops of Standards solution.
- Recap or snap down the pour spout on the Standards bottle quickly after dispensing. This is to prevent evaporation, which will change the osmolality of the solution, and to prevent microbiological contamination.
- Discard the sample tubes containing Standards after calibration or a QC process. This is to ensure fresh standards are used for every procedure. Never return a Standard to the bottle.

Discard Standards bottles when:

- There is evidence of turbidity or other microbiological contamination.
- Crystals appear on the outside of the bottle or ampule.
- The current date is past the expiration date on the bottle.
- Only 30 percent of the contents of the bottle remains.

Open the Precision Systems CON-TROL Standards glass ampules carefully. To use, pour directly from the ampule into the Precision Systems disposable test tube. Never pipette directly from the ampule. Discard the ampule after one use; never save and reuse an open ampule.

C. Quantimetrix Controls Principle

The Quantimetrix Human Urine Controls are intended as a means of monitoring human urine osmolality results to validate quantiation of patient samples. Control materials having known component concentrations are an integral part of diagnostic procedures. Daily monitoring of control values establishes intralaboratory parameters for accuracy and precision of the test method.
The typical precision of the OSMETTE may be expected to be <2 mOsm/kg H_2O for 1 SD for serum, urine, and salt standards for concentrations below 500 mOsm, and <1.0% for readings above 500 mOsm.

D. Quantimetrix Controls Storage and Handling

The Quantimetrix Human Urine Controls are supplied as a ready-to-use liquid requiring no reconstitution. They are prepared from human urine. The Human Urine Controls are fortified to

target levels with human proteins and reagent grade chemicals. Preservatives have been added to inhibit microbial growth.

Though the Quantimetrix Human Urine Controls have been tested nonreactive for Hepatitis and HIV antibodies, no known test product can assure that a product derived from human blood does not contain these viruses. Therefore, the controls should be handled according to the Centers for Disease Control's Biosafety Level 2 recommendations.

Always:

- Treat the controls as an SP sample.
- Store the controls refrigerated at 2 – 8 C. When stored at this temperature, the controls are stable until the expiration date stated on the label.
- Use only the amount of solution needed to gain acceptable pipetting technique and results. Under normal circumstances, this will be about two to three drops of Standards solution.
- Invert the bottle gently to ensure homogeneity of the solution before dispensing. Avoid foaming.
- Dispense the Quantimetrix controls into a Precision Systems sample tube before running the sample. Be sure to label the sample tube using a permanent marker.
- Recap the Quantimetrix control bottle immediately after dispensing the test amount and promptly return the bottle to the refrigerator.
- Allow the sample to sit for 30 minutes to come to room temperature before running the control sample.

Discard the controls when:

- The controls are past the marked expiration date.
- The control has been opened and it is 24 months from the date of manufacture or past the expiration date, whichever comes first.
- The control displays signs of turbidity.
- There is any evidence of microbial contamination.

E. Proficiency Testing

Evaluation and participation in the College of American Pathologists (CAP) proficiency-testing program is part of the comprehensive quality control program. The CAP samples for osmolality are shipped three times per year. Each Urine Chemistry (General) "U" shipment includes six 15.0-mL urine specimens. Follow all CAP instructions in preparing the materials before performing the test. Run specimens in a manner identical to routine specimens. Fill out the CAP result form, make a copy for the logbook, and send results to CAP.

F. Linearity for Osmolality

CAP LN6 verifies the reportable range of the Precision Osmette II instrument. Survey LN6 corresponds to CAP Urine Chemistry (General) Survey U. Each shipment of Survey LN6 includes 15 4.0-mL liquid specimens. Linearity was performed at installation and will be repeated in November of each year. The medical technologist follows all CAP instructions in preparing and running the materials before performing the test. They fill out the CAP result form, make a copy for the logbook, send results to CAP, and send the copy back to the home office at the end of the stand.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

If a sufficient, acceptable quality sample is submitted for osmolality testing, the medical technologist should run the sample twice. The two runs should have a difference no greater than 40, and the average of the two runs will be saved to the database as the osmolality result. The computer application will automatically select runs that it evaluates to meet the criteria for saving to the database. However, it is ultimately the responsibility of the medical technologist to evaluate each run and determine whether the result should be used in the average and save to the database. The application will not prevent any result from being used in an average if selected by the medical technologist, or any two results selected by the medical technologist from being saved to the database.

12. LIMITATIONS OF METHOD: INTERFERING SUBSTANCES AND CONDITIONS

A. Specimens contaminated with blood should not be analyzed

B. Specimens which are cloudy, or appear contaminated with bacteria should not be analyzed.

C. Particulate matter will cause the sample to pre-seed when supercooled, interrupting the measurement cycle. Undissolved particles or precipitate in the sample may be eliminated by centrifuging the sample.

13. REFERENCE RANGES (NORMAL VALUES)

50-1400 mOsm/kg H_2O.

14. CRITICAL CALL RESULTS (PANIC VALUES)

Not applicable.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

A. Specimen Storage

1. Store specimens refrigerated until processed.

2. Run within 4 hours after collection.

16. ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

There is no alternative method for this test.

Store urine in the refrigerator for no more than 4 hours.

17. TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

All records, including QA/QC data, will be maintained for 6 years.

Use only numerical identifiers for SP results.

18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

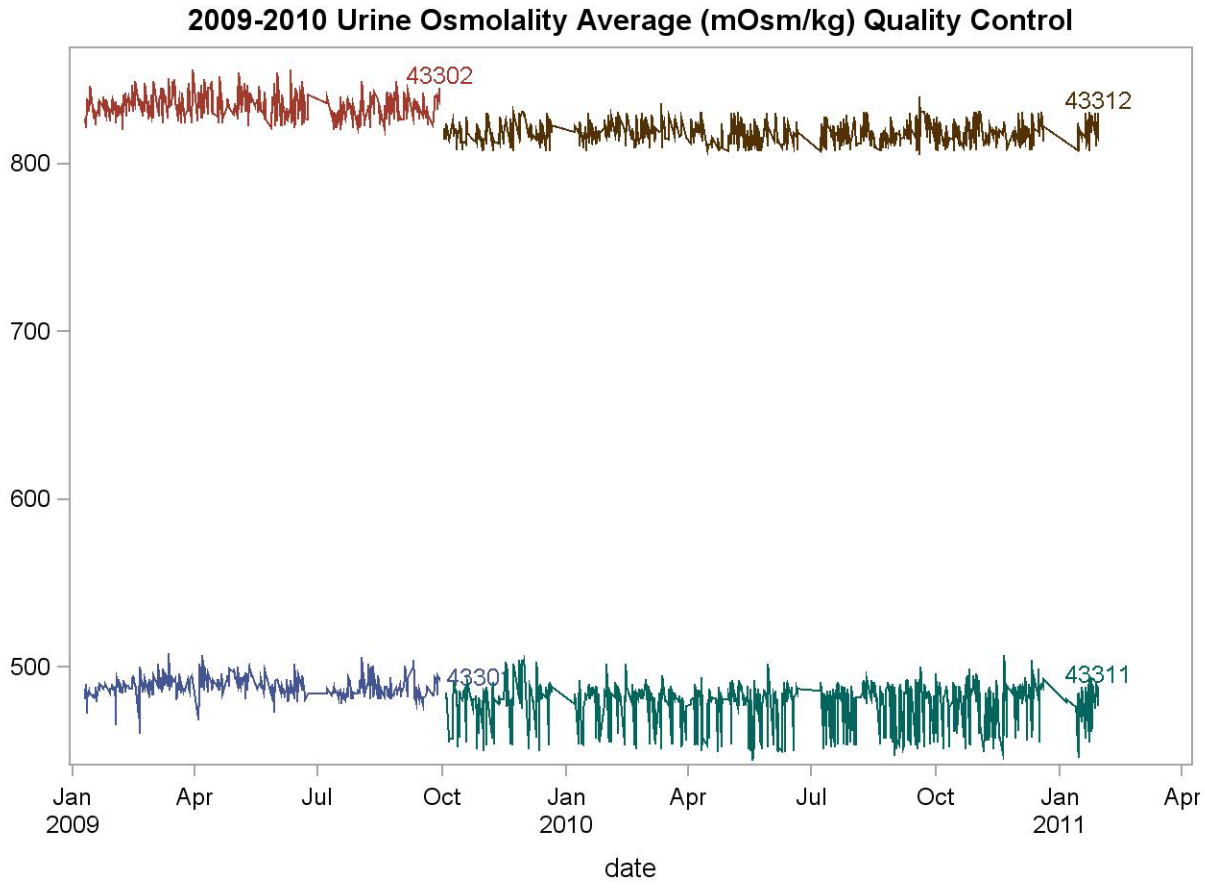
In general, when specimens are received, the specimen ID number, and a name identifying the container ID and slot number is entered into the Integrated Survey Information System (ISIS) database. New barcodes are printed and the specimens are analyzed within 2 hours in the MEC. The specimen ID is scanned by a barcode reader. The date and time of data entry, date and time analysis completed, and the identity of the person who accepts or rejects the results, are documented in the ISIS database.

Logs are kept which include the date and time samples arrive, are processed, tested, frozen, and returned to NHANES for long term storage.

The Project supervisor is responsible for keeping a logbook containing the ID numbers of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, with information about these discrepancies. It is recommended that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study.

19. Quality Control Summary Statistics and Graphs

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
43301	371	10JAN09	29SEP09	488.3	6.0	1.2
43302	455	10JAN09	29SEP09	833.8	7.3	0.9
43312	780	03OCT09	29JAN11	817.6	6.4	0.8
43311	860	04OCT09	29JAN11	478.4	13.0	2.7



20. References

Precision Systems Osmette II Operating Manual Model 5005, Precision Systems, Inc. 2006

Osmette II™ (Model 5005 Automatic Osmometer) Operating Manual, Part Number 2095. Precision Systems Inc. 2006.