

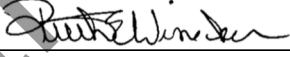


SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

Table of Contents

1. Principle of Assay	3
2. Specimens	4
3. Reagents and Materials	4
4. Standards, Controls, and Solutions	4
5. Instrumentation and Equipment	4
6. Procedure for Assay	4
7. Calculations.....	11
8. QA/QC	11
9. Validation of Method	12
10. Reporting	12
11. Preparation of Load	13
12. References	13

SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

SOP Name: Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer		SOP #: 110
North Carolina Office of the Chief Medical Examiner Toxicology Laboratory	Revision:	Revision Date/Initials:
	6.1.3 – Added pre-run maintenance instruction	MSF – 12/06/2017
Approving Authority Name	Approving Authority Signature	Approval Date
Ruth E. Winecker, Ph.D.		04/08/2015
Ruth E. Winecker, Ph.D.		06/10/2016
Ruth E. Winecker, Ph.D.		12/07/2017

SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

1. Principle of Assay

- 1.1. The measurement of glucose, electrolytes, creatinine, and urea nitrogen in vitreous fluid can be a helpful diagnosis tool in the determination of means and manner of death in Medical Examiner cases. Compartmentalized within the eye, vitreous humor is less susceptible to postmortem chemical changes caused by the breakdown of cell membranes and other factors making it a superior specimen to postmortem whole blood.
- 1.2. The diagnostic uses of each of the measured compounds are as follows:
 - 1.2.1. Sodium – used in the diagnosis of aldosteronism, diabetes insipidus, adrenal hypertension, Addison’s disease, dehydration, or diseases involving electrolyte imbalance.
 - 1.2.2. Potassium - used in the diagnosis of electrolyte imbalance and disease conditions characterized by low or high potassium levels.
 - 1.2.3. Chloride - used in the diagnosis of electrolyte and metabolic disorders such as cystic fibrosis and diabetic acidosis.
 - 1.2.4. Urea Nitrogen - used in the diagnosis of certain renal and metabolic diseases.
 - 1.2.5. Creatinine - used in the diagnosis of renal conditions.
 - 1.2.6. Glucose - used in the diagnosis of diabetes mellitus,.
- 1.3. Chemical Sensors
 - 1.3.1. Sodium, Potassium, and Chloride electrolytes require a sensing ion selective membrane, a reference electrode, and an internal filling solution. The Internal filling solution provides a fixed concentration of the ion to which the specimen is measured by comparison.
 - 1.3.2. Urea Nitrogen measurement uses urease which has been chemically bonded to a membrane to catalyze the conversion of urea present in the sample to ammonia and CO₂. An ammonium ion selective electrode is used to detect the ammonium formed by this reaction.
 - 1.3.3. Creatinine measurement uses 3 sensors to catalyze the conversion of creatinine to form formaldehyde, glycine, and hydrogen peroxide. The amount of hydrogen peroxide formed is proportional to the amount of creatinine in the sample.
 - 1.3.4. Glucose measurement is based on the level hydrogen peroxide produced during the enzymatic reaction between glucose and oxygen molecules in the presence of the glucose oxidase enzyme.

SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

2. Specimens

- 2.1. This procedure is applicable to **non-embalmed** vitreous humor (0.3mL). In order to decrease viscosity, it may be necessary to treat samples with hyaluronidase.
 - 2.1.1. To treat specimen with hyaluronidase, aliquot 500µL of specimen into 75 x 100mm test tube. Add ~10mg hyaluronidase and vortex mix for 20 seconds. Place into centrifuge for 5 minutes @ 2000 x g. Transfer sample to 0.5mL sample cup for analysis.
 - 2.1.2. Any vitreous humor specimen that appears discolored (bloody), decomposed, or embalmed should not be analyzed. Report the specimen as “Electrolytes – Specimen unsuitable” with a result comment “Specimen matrix was unsuitable for glucose and electrolytes analysis”.

3. Reagents and Materials

- 3.1. Calibrator Cartridge 1 with Creatinine
- 3.2. Chemistry Controls Cartridge 3 with Creatinine
- 3.3. Sodium Sensor
- 3.4. Potassium Sensor
- 3.5. Chloride Sensor
- 3.6. Glucose Sensor and Membrane Cap
- 3.7. BUN Sensor and Membrane Cap
- 3.8. Creatinine Sensor and Membrane Cap
- 3.9. Deionized Water
- 3.10. 100-1000 µL Pipettor with tips
- 3.11. 0.5mL sample cups (or equivalent)
- 3.12. 75 x 100mm test tubes
- 3.13. Hyaluronidase from bovine testes

4. Standards, Controls, and Solutions

- 4.1. N/A

5. Instrumentation and Equipment

- 5.1. Stat Profile pHox Ultra Analyzer

6. Procedure for Assay

- 6.1. Initial Instrument Setup, Maintenance, and Calibration:
 - 6.1.1. To activate the analyzer’s interface, touch anywhere on the screen.
 - 6.1.2. Log in: touch the “Key” icon in the upper left corner of the screen.

SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

- 6.1.2.1. Touch the “User Name” field and enter your user name using the on-screen keyboard. Touch ‘Enter’.
- 6.1.2.2. Touch the “Password” field and enter your password using the on-screen keyboard. Touch ‘Enter’.
- 6.1.2.3. Select “Log on”
 - 6.1.2.3.1. If you do not have a user name and password, contact the QC/QC chemist to set one up.
- 6.1.3. Replace Pump Tubing (monthly) as outlined on page **3-18** of the Stat Profile pHox Ultra Analyzer Instructions for Use Manual
- 6.1.4. Replace the ABG/Chem Calibrator Cartridge 1 as outlined on page **3-20** of the Stat Profile pHox Ultra Analyzer Instructions for Use Manual **injecting the contents of each syringe into the matching colored ports on the back of the cartridge prior to installation.**
 - 6.1.4.1. Replace the Capillary Adapter (replacement is found in the ABG/Chem Calibrator Cartridge 1 box) as outlined on page **3-19** of the Stat Profile pHox Ultra Analyzer Instructions for Use Manual
- 6.1.5. Replace the ABG Control Pack 3 as outlined on page **3-22** of the Stat Profile pHox Ultra Analyzer Instructions for Use Manual **injecting the contents of each syringe into the matching colored ports on the back of the cartridge prior to installation.**
- 6.1.6. Replace the Glucose, BUN, and Creatinine Membrane caps as outlined on page 3-8 & 3-9 of the Stat Profile pHox Ultra Analyzer Instructions for Use Manual.
- 6.1.7. Replace Cl⁻, Na⁺, K⁺ sensors as needed as outlined on page 3-7 of the Stat Profile pHox Ultra Analyzer Instructions for Use Manual and according to the Maintenance Schedule Log located next to the instrument.
- 6.1.8. Wait a minimum of 30 minutes to allow the new sensor membranes to equilibrate.
- 6.1.9. Condition Flow Path with Blank Blood
 - 6.1.9.1. Choose “System” – “Maintenance”
 - 6.1.9.2. Choose “Condition Flow Path” by touching the adjacent box - Touch “Start”.
 - 6.1.9.3. Present Blank Blood to sample Probe and touch “Aspirate”
 - 6.1.9.4. When prompted, remove blank blood from probe and touch “Continue” (5min).

SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

- 6.1.10. Calibrate the instrument - touch the “Clock” icon on the upper right part of the screen.
 - 6.1.10.1. Choose “ABG/Chem Calibration” by touching the adjacent box. Touch “Start” (5min).
 - 6.1.10.1.1. Multiple calibrations may be required in order to obtain acceptable QC results for some analytes. Do not start analyzing QC/Specimens until two consecutive successful calibrations have been achieved.
- 6.1.11. Run Performance Check – Touch “System” – “Run QC”
 - 6.1.11.1. Select the “Performance Check” tab.
 - 6.1.11.2. Under “Internal QC Level” dropdown, select “Perf Chk” and touch “Start”.
 - 6.1.11.3. Touch “Home” icon when finished.
- 6.1.12. Once all of these steps are completed, all of the test icons should be blue. If any are orange, touch the icon for that test and complete the suggested corrective action.
- 6.1.13. If corrective action is unsuccessful, ask a senior chemist for assistance.
- 6.2. QC
 - 6.2.1. Update instrument QC acceptance ranges for Level 4 and level 5:
 - 6.2.1.1. Use the “QC Ranges – Calculator” to calculate the low and high acceptance ranges for each analyte in QC level 4 & 5 based on target concentrations found on QC product insert and record on the pHox Ultra “QC Ranges worksheet”. <S:\toxicology\Instrument Logs and Manuals\Instrument Maint. logs\pHox Ultra Analyzer>
 - 6.2.1.2. Select “System” → “Setup” → “QC”, and choose the “Ranges” tab.
 - 6.2.1.3. Enter ranges into the pHox Ultra and select “Save” → “Save to all instruments”.

SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

Analyte	Acceptance Range (Lev 4)	Acceptance Range (Lev 5)
Na ⁺	±7.5%	±7.5
K ⁺	±7.5	±5.0
Cl ⁻	±7.5%	±7.5
Glucose	±20%	±20
Creatinine	±50%	±40
Urea Nitrogen (VUN)	±25	±12.5

6.2.1.4.

6.2.2. QC's (levels 4 and 5) shall be analyzed before the analysis of case specimens begins as well as following the completion of case specimens.

6.2.3. From the Home Screen, Touch "System" – "Run QC".

6.2.4. Select QC level 4 from the "Internal QC" drop-down menu and touch "Start"

6.2.5. After the analysis is complete, the results will be displayed on screen.

6.2.5.1. Review the results to verify all are within acceptance limits.

6.2.5.2. If any results are out of range, re-calibrate and reanalyze.

6.2.6. Repeat steps outlined above (6.2.2– 6.2.5) for QC level 5.

6.2.6.1. **Note – During specimen analysis, it may be necessary to recalibrate the instrument due to matrix effects (6.1.10). All recalibrations shall be followed by the analysis of QC level 4 to validate calibration.**

6.3. Specimen Analysis

6.3.1. Aliquot 0.3mL of specimen into a 0.5mL sample cup taking care not to take any bits of tissue or other debris that may be present.

6.3.1.1. Make a note on the "Specimen Locate Sheet" if a specimen matrix shows signs of discoloration or decomposition.

6.3.1.1.1. Cloudy

6.3.1.1.2. Discolored

6.3.1.1.3. Bloody

6.3.1.1.4. Decomposed

6.3.2. On the Home Screen, touch the Patient ID field and enter the last 1-5 digits of the S#.

6.3.3. Touch the "Additional Information" button.

SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

- 6.3.4. In the “Accession Number” and “Account Number” fields, enter the full S# and T# respectively, pressing “Enter” after each. Touch “Save”.
 - 6.3.5. On the Home Screen, touch start.
 - 6.3.6. Following the on screen directions, present sample to the sample probe and touch the “Aspirate” button.
 - 6.3.7. When prompted that sample aspiration is completed, remove the specimen from the sample probe and touch “Continue”. The probe will withdraw and the sample analysis will begin.
 - 6.3.8. After the analysis (2min) the results will be displayed on screen (and automatically printed).
 - 6.3.8.1. Review the results and check for error messages
 - 6.3.8.2. Drift or sample pathway errors may require the flow path to be reconditioned, the instrument re-calibrated, QC level 4 run, and the specimen reanalyzed.
 - 6.3.8.3. Some specimen matrices will cause the Creatinine sensor to become unstable resulting in a calibration error or “Creatinine Drift”. Follow the following steps to recalibrate Creatinine.
 - 6.3.8.3.1. Recondition the Flow Path with Blank Blood (6.1.9).
 - 6.3.8.3.2. Run system calibration (6.1.10).
 - 6.3.8.3.3. Run QC level 4 to verify successful calibration.
 - 6.3.8.3.4. Report creatinine as “Specimen Unsuitable” (Do not reanalyze specimen).
 - 6.3.8.4. Low and High Range errors are result based and can be expected in post mortem specimens
 - 6.3.9. Go back to the Home Screen (House Icon) and repeat steps 6.3.1– 6.3.8 for the remaining specimens.
 - 6.3.10. After all specimens have been analyzed, run QC levels 4 and 5 again following steps 6.2.2– 6.2.5.
- 6.4. Print Reports
- 6.4.1. To print a list of sensor and reagent lot numbers used, touch “Data” – “Reports” – “Maintenance Log Report” – “Print” (lower right).

SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

- 6.4.1.1. If all sensor and reagent change-outs were done the same day as analysis, leave “Today” as the time reference.
 - 6.4.1.1.1. If the instrument was set up on a previous day, choose the appropriate time range in the dropdown menu or choose a custom date range using the calendar buttons.
- 6.4.1.2. Touch “Print”, the report will be displayed in PDF format. Touch the printer icon on the upper left and select “Print”.
- 6.4.1.3. Close the report by touching the “Close” button (top center).
- 6.4.2. To print an instrument calibration history, touch “Data” – “Reports” – “Calibration Report” – “Print” (lower right).
 - 6.4.2.1. If the instrument was set up on the same day as analysis, leave “Today” as the time reference.
 - 6.4.2.1.1. If the instrument was set up on a previous day, choose the appropriate time range in the dropdown menu or choose a custom date range using the calendar buttons.
 - 6.4.2.2. Touch “Print”, the report will be displayed in PDF format. Touch the printer icon on the upper left and select “Print”.
 - 6.4.2.3. Close the report by touching the “Close” button (top center).
- 6.4.3. Print QC Results: (QC results should print automatically following analysis – if for some reason additional reports are needed, follow the steps below).
 - 6.4.3.1. Select “Data” – “Reports” – “QC” – “Print”.
 - 6.4.3.2. Time Range should read “Today” – “Print”.
 - 6.4.3.3. Touch box adjacent to desired test (start at the bottom for oldest QCs) and select “Single Result” – “Print”.
 - 6.4.3.4. Choose “Printer by Kim” from the dropdown menu and “Print”.
 - 6.4.3.5. Repeat steps 6.4.3.1– 6.4.3.4 for all QC level 4 and 5 results.
- 6.4.4. Print Case Reports: (Case results should print automatically following analysis – if for some reason additional reports are needed, follow the steps below).
 - 6.4.4.1. Select “Data” – “Reports” – “Patient Data” – “Print”.
 - 6.4.4.2. Choose the appropriate time range (Today) – select “Print”.

SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

- 6.4.4.3. Touch the box adjacent to desired Specimen (start at the bottom for earlier analyzed cases) and select “Single Result” – “Print”.
- 6.4.4.4. Choose “Printer by Kim” from the dropdown menu and “Print”.
- 6.4.4.5. Repeat steps 6.4.4.1– 6.4.4.4 for all specimens.
- 6.4.4.6. **Collect reports from printer. Check to be sure all reports were printed properly and are accounted for before moving on to next step.**

6.5. Prepare Data for upload (pending – skip to 6.6 until further notice)

Note: The following procedure will permanently remove case data from the instrument.

- 6.5.1. Plug USB thumb drive labeled “pHOx Ultra” into one of the two USB ports located on the left rear of the instrument.
- 6.5.2. Select “System”, “Database”.
- 6.5.3. Choose “Archive Patient Data”.
 - 6.5.3.1. The time range should read “Older than -01 Days”.
 - 6.5.3.2. Select the file to which the data is to be transferred: D: “month/year of analysis” e.g. D: \August 2013
 - 6.5.3.3. Choose “Select”
- 6.5.4. Touch “Start” – after a few moments the message “Archive Patient Data Completed Successfully”
- 6.5.5. Remove thumb drive from the instrument and plug it into a networked PC.
- 6.5.6. Copy the folder containing that day’s archived data to S:\toxicology\Instrument Data\pHOx Ultra.

6.6. Housekeeping

- 6.6.1. When all analysis is complete and data transferred, touch the “Clock” icon and select “Enter Standby”. Click on the “X” to close the window.
- 6.6.2. Touch the “Key” icon and Select “Logout”.

SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

6.6.3. Dampen a few Kim-wipes with 10% bleach solution and wipe down the touch screen. Wait 30 seconds and dry the screen with dry Kim-wipes.

6.7. Upload Data to Toxlog – Procedure Pending

7. Calculations

7.1. The pHox Ultra uses different types of sensors to detect and quantitate the various analytes of interest – see attached for details.



Adobe Acrobat
Document

8. QA/QC

8.1. A successful performance check must be analyzed within 24 hours prior to analysis

8.1.1. The performance check evaluates the ability of the instrument to differentiate between glucose vs. similar molecules and creatinine vs. similar molecules.

8.1.2. Without a passing performance check, analysis of glucose and creatinine is not possible.

8.2. A successful two-point calibration must be performed prior to the analysis of case specimens.

8.2.1. If a two-point calibration does not meet manufacturer determined criteria for an analyte, the instrument will not analyze for that analyte until a successful calibration is performed.

8.3. The instrument performs a 1-point calibration check during every QC and case specimen analysis.

8.3.1. If the calibration check does not meet manufacturer determined criteria for an analyte, a “drift” error will occur and no results will be displayed for that analyte until recalibration is performed and specimen repeated.

8.4. QC level 4 and 5 are to be analyzed both prior to and following case analysis and must pass acceptance criteria.

8.4.1. Upon QC failure, recalibration is allowed as the sample matrix of the previous specimen may have caused sensor instability.

8.5. Reporting Results with Warning Flags:

SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

- 8.5.1. Occasionally, specimen matrix will cause instrument errors to be flagged (e.g. “AD4 – Insufficient Sample”). As long as results have been calculated for each analyte, meaning sufficient specimen had passed by the various detectors, these error messages can be labeled as “N/A” and ignored.

9. Validation of Method

- 9.1. Validation consisted of comparisons of 22 case specimens analyzed by UNC Hospital and NMS Laboratories and subsequently analyzed using the pHox Ultra analyzer
- 9.2. Results were consistent and provided similar toxicological interpretations
- 9.3. Validation results are located: S:\toxicology\QAQC\Method Validation\pHox Ultra

10. Reporting

10.1. Units

- 10.1.1. Sodium (Na^+), Potassium (K^+), and Chloride (Cl^-) shall be reported in **mmol/L**.
- 10.1.2. Glucose, Creatinine, and Vitreous Urea Nitrogen (VUN) shall be reported in **mg/dL**.

10.2. High range results shall be reported as “Greater Than ###”

10.3. Low range results shall be reported as “Less Than ###” (see Table 1 below).

10.4. Specimens that displayed signs of discoloration/decomposition (as described in 6.3.1.1) shall receive an appropriate ‘Specimen Comment’ (see 10.5) such as:

- 10.4.1. Specimen matrix appears cloudy
- 10.4.2. Specimen matrix appears discolored
- 10.4.3. Specimen matrix appears bloody
- 10.4.4. Specimen matrix appears decomposed

10.5. Specimen comments must be entered in MEIS by authorized personnel.

- 10.5.1. Login to MEIS and select ‘Tox Administration’ from the menu (top).
- 10.5.2. Under ‘Tox Admin’ (left) select the ‘Update ToxSpecimen’ button.
- 10.5.3. Enter the Toxicology folder number (T#) and select ‘Find’.
- 10.5.4. Choose (click on) the appropriate Specimen number.
- 10.5.5. Select the “Other” radio button and type or copy/paste the specimen comment (10.4) into the text field and click ‘Update’.

10.6. Analyte Measurement Ranges:

10.6.1.

SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

Analyte	pHOx Ultra Linear Range	Normal (Postmortem Vitreous)
Na ⁺	80 - 200 mmol/L	135 - 150 mmol/L
K ⁺	1.0 - 20.00 mmol/L	<15 mmol/L*
Cl ⁻	50 - 200 mmol/L	105 - 135 mmol/L
Glucose	15 - 500 mg/dL	<200 mg/dL**
Creatinine	0.2 - 20.0 mg/dL	0.6 - 1.3 mg/dL
Urea Nitrogen (VUN)	3.0 - 100 mg/dL	8 - 20 mg/dL

* Levels increase postmortem

**Levels decrease postmortem

Table 1

11. Preparation of Load

11.1. Upon completion of the analysis, the analyst should verify that all specimen data has been collected. Once the calculations performed by the CO-Oximeter software have been reviewed, in addition to all clerical work, the analyst will assimilate the data in the following order: (All documentation should include the appropriate load number).

- 11.1.1. Assignment sheet
- 11.1.2. Applicable note-to-file documentation
- 11.1.3. Load summary
- 11.1.4. Chain of custody
- 11.1.5. Aliquot chain of custody
- 11.1.6. Standards/control worksheet (Calibration and control kit inserts)
- 11.1.7. Maintenance log & Calibration log reports
- 11.1.8. Calibration and quality control.
- 11.1.9. The individual case reports are included last.

11.2. The finished data package will be placed in the data review box in the office area of the toxicology laboratory (room 2401).

12. References

- 12.1. Garg U, Althahabi R, Amirahmadi V, Brod M, Blanchard C, Young T. Hyaluronidase as a liquefying agent for chemical analysis of vitreous fluid. *J Forensic Sci.* Mar 2004;49(2):388-91.

SOP 110 - Glucose, Electrolytes, Creatinine, and Urea Nitrogen in Post Mortem Vitreous Humor Using the pHox Ultra Chemical Analyzer

- 12.2. Rose KL, Collins KA. Vitreous postmortem chemical analysis. NewsPath [serial online]. December 2008;1-3. College of American Pathologists. Available at <http://bit.ly/f2t2FL>. Accessed June 7, 2010.
- 12.3. *Nova Biomedical Stat Profile pHox Ultra Analyzer Instructions for Use Manual*. 2011. User Manual. USA, Waltham, MA.
- 12.4. *Nova Biomedical Stat Profile PHOx Ultra Analyzer Advanced User Applications Manual*. 2011. User Manual. USA, Waltham, MA.

Uncontrolled Copy