

**FORENSIC TOXICOLOGY LABORATORY
OFFICE OF CHIEF MEDICAL EXAMINER
CITY OF NEW YORK**

VITREOUS HUMOR CHEMISTRIES

PRINCIPLE

Post mortem blood concentrations of certain substances (e.g., glucose, electrolytes, creatinine, BUN/urea) do not necessarily reflect ante mortem concentrations shortly prior to death. Vitreous humor concentrations of such substances provide a more accurate interpretation.

The Pentra C200 system is a fully automated chemistry analyzer. It has been designed to measure enzymes, substrates, specific proteins and ions by using colorimetry, turbidimetry and potentiometry technologies. The instrument is designed for homogeneous samples such as serum, plasma and urine. Vitreous humor has also been demonstrated to be an acceptable sample.

The instrument is composed of a reagent storage module, a sampling module, an ISE module and an additional module for glucose, urea nitrogen and creatinine.

SAFETY

The handling of all reagents, samples and equipment is performed within the guidelines which are detailed in the safety manual.

REAGENTS AND MATERIALS

Horiba ABX MCal (lyophilized material or liquid form) or equivalent
Horiba ABX N control (lyophilized material or liquid form) or equivalent
Horiba ABX P control (lyophilized material or liquid form) or equivalent
Horiba ABX L urine control (liquid material) or equivalent
Horiba ABX H urine control (liquid material) or equivalent
Horiba ABX Standard 1 (ISE) or equivalent
Horiba ABX Standard 2 (ISE) or equivalent
Horiba ABX Reference (ISE) or equivalent
Horiba ABX Qualitest solution or equivalent
Horiba ABX Etching CP or equivalent
Horiba ABX Deproteinizer CP solution or equivalent
Horiba ABX Creatinine CP or equivalent
Horiba ABX Glucose CP or equivalent
Horiba ABX Urea CP or equivalent
Human Serum from UTAK or equivalent
Biocups (sample cups)
Automated pipettor with tips
Pasteur pipet (5 3/4")
Volumetric pipettes (3mL, 5mL)
Distilled or reagent grade water
Bleach (10% solution diluted with distilled water)

REAGENT PREPARATION

MCal – Reconstitute the contents of one vial with 3 mL of distilled or reagent grade water using a 3 mL volumetric pipet. Be careful when opening the rubber cap as some material may have adhered to the cap. Close the vial and place it in a refrigerator. **Allow to stand overnight.** The next day, invert gently and make sure all of the material has been completely dissolved. **Do not shake.** Aliquot into a labeled biocup (date, lot number and preparer's initials) as needed. Store the balance of the reagent in the refrigerator. The MCal solution is stable for 1 week from reconstitution date.

N control – Reconstitute the contents of one vial with 5 mL of distilled or reagent grade water using a 5 mL volumetric pipet. Be careful when opening the rubber cap as some material may have adhered to the cap. Close the vial and place it in a refrigerator. **Allow to stand overnight.** The next day, invert gently and make sure all of the material has been completely dissolved. **Do not shake.** Aliquot into a labeled biocup (date, lot number and preparer's initials) as needed. Store the balance of the reagent in the refrigerator. The N control solution is stable for 1 week from reconstitution date.

P control – Reconstitute the contents of one vial with 5 mL of distilled or reagent grade water using a 5 mL volumetric pipet. Be careful when opening the rubber cap as some material may have adhered to the cap. Close the vial and place it in a refrigerator. **Allow to stand overnight.** The next day, invert gently and make sure all of the material has been completely dissolved. **Do not shake.** Aliquot into a labeled biocup (date, lot number and preparer's initials) as needed. Store the balance of the reagent in the refrigerator. The P control solution is stable for 1 week from reconstitution date.

*NOTE: Newly reconstituted MCAL, N control and P control **must stand overnight.** Check the day before that there is a sufficient amount of in date MCAL, N control and P control to run the analyzer.*

INSTRUMENTATION

Pentra C200 chemistry analyzer with an embedded Window XP computer, Printer
ISE module (chloride, potassium and sodium ion electrodes)
Water and waste containers
Cuvettes
Waste bags
Centrifuge
Water filter

INSTRUMENT SETUP

1. At the beginning of any day of use, the instrument must be switched from sleeping mode to Ready. When the instrument is on sleeping mode, the green LED on the right of the screen blinks and the screen is black. During automatic wake up the instrument performs initialization. When the preparation process is finished the status changes from “start up” to “ready” and the pop-up disappears. Press the shutdown icon on the generic tool bar at the top of the main screen and select login from the drop down menu. When the login

screen appears, select the user name from a dropdown menu and enter your password. Press login. A pop-up is displayed for user confirmation. Press OK. Press the Back button on the generic toolbar to enter the main screen.

2. The instrument must be powered on at all times to provide constant refrigeration for reagents. If the instrument is not going to be used for two or more consecutive days, take the reagent tray and place it in the refrigerator.
3. Remove creatinine cassette before placing reagent tray in the refrigerator.

*NOTE: The creatinine cassette **must** be stored at room temperature*

4. Make sure the sample and reagent container unit cover (**SRCU**) and main lid cover of the instrument are closed while in sleep mode and during analysis.
5. As needed, remove reagent tray from the instrument to load reagent cassettes into a numbered position on the tray. Click on the cassette icon on the upper tool bar. The reagent tray tab displays a representation of the reagent tray and cassette information. Each cassette is symbolized by two circles for outer compartment and inner compartment. To access information about a cassette, press the appropriate circle on the tray display. The information about the cassette compartment is shown in the cassette information area. See illustration in the Pentra C200 User's manual page 20. The reagent tray holds the reagent cassettes and the sample tray fits perfectly over the reagent tray,

NOTE: The user's manual can also be found online in the instrument by selecting the “? Icon” on the top of the tool bar.

6. Use a paper towel to dry condensation from the refrigerated compartment of the instrument before placing the reagent in the compartment. Once positioned cover with the SRCU cover.
7. Check volumes for ISE **Standard 1** and ISE **Reference** reagents.. Replace bottles if volume is lower than 28 mL for **Standard 1** and 10 mL for **Reference**. To change the ISE solutions, open the main cover. Open the ISE solution compartment cover. Unscrew and remove each cap with tubing and straw from bottle and dry the straw. Remove the bottles from the ISE solution compartment. Install the new bottles. Screw each cap with tubing and straw onto its corresponding new reagent bottle. Close the main cover.
8. **Reset the bottle volume.** To reset the bottle volume, go to “Services”, “Customer Services”, “Workhour” tab. Press reset for the L solution (Standard 1) or Ref solution (Reference).
9. Prime the tubing with new solutions. To refill, go to “Services”, “Customer Services”, “Sequence” tab. Type 10 on the right of the ISE prime button to run 10 primes. Press the ISE prime. Wait until the end of the primes (instrument status changes from analyzing to ready). Open the main cover. Check that there is no leakage at the connections. Close the ISE solution compartment cover.. Refer to User's manual page 102.

NOTE: Use the green button on top of the tool bar to navigate backwards on any screen. Pressing the green button will ultimately take you to the main screen menu where all the functionality of the instrument is located. See diagram on User's manual page 17.

10. Check the number of cuvettes at the beginning of each day and after each run. There are 96 cuvettes in each rack. The instrument takes two racks at a time or 192 cuvettes. Click on the “Cuvette icon” located at the bottom left corner of the main screen menu to display a cuvette counter window. If more cuvettes are needed, put in a new rack of cuvettes. Select position **A1** for a full rack of cuvettes. Select any other position that coincides with the next available cuvette. The instrument will verify cuvette quality before each reaction. Refer to User’s manual page 24.

NOTE: Partially used racks of cuvettes may be rearranged but make sure that the bottom of the cuvette is not touched as it must be clear for optical measurements.

11. Prior to analysis empty the cuvette’s waste compartment. Remove the waste bin and take out the biohazard bag. Discard the biohazard bag and replace with a new one.
12. Refill the two water containers located inside the instrument. The first container is right next to the needle. Add distilled or reagent grade water to the mark on the outside of the plastic container. The second container is located right under the mixer. To perform this step, close the main lid of the instrument. Go to the main screen menu and select the services icon. Then select toolman icon and “mix ascend” from the menu. The mixer will ascend shortly thereafter. Open the main lid cover, remove the water container. Add 3 drops of the Qualitest solution and fill with distilled or reagent grade water to the mark on the outside of the plastic container. Place the plastic container back into position and close the main lid cover. From the same screen, select “mix descend”.
13. Check water container located outside of the instrument on a daily basis and at the beginning of each analysis. Refill as needed with distilled water or reagent grade water.

NOTE: Once you remove the tubing and sensor from the water container an alarm will sound. This is normal.

14. Empty water/reagent waste container prior to starting the analyzer.

START UP PROCEDURE

1. Prior to setting up a run, login to the instrument using your assigned username and password if you haven’t already done so. Select your **username** from the dropdown menu (position # 1). and type your **password** (position # 2). Select **login** (position # 3). Refer to User’s manual page 16.
2. On the main screen menu (User’s manual page 17), click the background outside of the reagent circle to view a legend of color status for reagents (empty, OK, to be checked, low volume).
3. On the main screen menu, select the reagent cassette icon, located on the toolbar at the top of the instrument. (Icon# 4 from left side)
4. Click on the outer circle of each reagent on the reagent tray to view detailed information about a particular reagent. This will indicate how many tests are left. If more reagent(s) is/are needed, then load a new reagent cassette onto the corresponding reagent tray. Refer to User’s manual page 18.

5. If a new reagent was loaded, perform a barcode reagent scan. To perform this step, go to the main screen menu. Press the cassette icon. A pop-up window will open. Select barcode scan from the bottom of the window. It will take 30 seconds for the instrument to complete the barcode scan.

NOTE: This pop-up window will give you access to reagent information.

6. Aliquot Standard 2 solution to a biocup. Proceed to calibrate the ISE module. From the instrument's main screen menu, click on the worklist icon. Select position # 1. Place the ISE **Standard 2** biocup in position # 1 of the sample tray. Go to "**Type**" and select ISE Calibration from the dropdown menu. Using the back arrow on the tool bar, go to main screen menu and click on "Services", the second icon on the right side of the circle. Click on the "Toolman" icon on the left side. Select ISE calibration from dropdown menu. The calibration will start right away. It will take about 7 minutes to complete calibration.

NOTE: ISE calibration must be performed at the beginning of each work day and every 8 hours. For more detailed information refer to User's manual page 25.

7. Print out calibration report. To perform this step, press the ISE icon located at the bottom right of the main screen menu. Press the print screen button on the tool bar for **two** seconds. Evaluate ISE calibration report. There will be four zeros in the error code box. This is normal. If other numbers or letters appear on this box, please perform ISE cleaning and run calibration again. If problem continues, contact Horiba Medical.
8. As needed, proceed to calibrate glucose, urea and creatinine by running a Horiba **MCal** calibrator. The instrument's main screen menu may display a red circle corresponding to a reagent position indicating that a reagent needs calibration. To find out which reagent, select the red circle and the reagent will be identified. This can be verified by selecting the test that needs calibration. The icon selected will also be red.
9. On the main menu select the worklist icon, the first icon on the left. On the selection tab, Select position # 1. This will give you two positions on the sample tray. Go to "**Type**" and select Multistandard. Go to "**Cup**" to select biocup. Go to "**Sample**" to select common. Go to "**MS**" to select MCal. Click on the test(s) that requires calibration. Click save.

NOTE: Once position # 1 is selected, position # 2 will automatically be created **except for urea analysis.**

10. Select position # 3, go to "**Type**". From the dropdown menu select control. Go to "**Cup**" to select biocup. Go to "**Control**" to select N control. Select requested tests. Click save.
11. Select position # 4, go to "**Type**". From the dropdown menu select control. Go to "**Cup**" to select biocup. Go to "**Control**" to select P control. Select requested tests. Click save.
12. Load samples in the sample tray as follows:

Position # 1 = **water**,

Position # 2 = **MCal**

Position # 3 = **N control**

Position # 4 = **P control**

If only calibrating for urea, a water is not needed in position # 1. The positions are then:

Position # 1 = **MCal**

Position # 2 = **N control**

Position # 3 = **P control**.

13. Close reagent/sample compartment and close the lid on the instrument. Press the start icon (first icon on the toolbar, top left corner of the main screen menu) for **two** seconds. A window indicating “check volume of detergent” will pop up. Select OK. This message will appear every time a sequence is run.

NOTE: A detergent wash is not needed for vitreous analysis.

Calibration and control reports will print automatically. Evaluate results from calibration and control. Make sure that the concentration for each analysis is within the manufacturer's established range. Affix an N control and P control target/range label. Sign and date the reports. Keep calibration and control reports together and place it in a binder. If control fails, a new calibration with a newly reconstituted MCal must be performed. Run the N and P controls to make sure the new calibration worked.

CALIBRATION & QUALITY CONTROL

1. In addition to the calibration and control print outs, results can be viewed under “**Run Results**”.
2. Results are displayed in the run screen as soon as the measurement is done.
3. Review calibration details by going to main screen menu, select “**Calibration button**”. A window will pop up.
4. Under the “**Reg Calib**” tab, you can view the calibration for a specific test. To view a different test, go to “**Method**” and select one from the dropdown menu. The following are the method codes for the analytes commonly run in this laboratory: Glucose = 22, Creatinine = 52, BUN/Urea = 32, Chloride = 1003, Sodium = 1001, Potassium = 1002,
5. Control review is performed from the main screen menu. Select the “**QC**” icon from the main screen menu. A window will pop up. Under the “**Details**” tab, select the method to view using the same method codes in step 4. Go to “**Sample**” and select “**Common**”. On the “**Result output**” select “**Monitor**” to see results on the screen; or select “**Print**” if a print out is needed. Results can also be saved to an external drive to view on a different computer. Select the “**Control name**” to see control results. (e.g. N control or P control) Select a specific date or a range of dates as needed.

For urine samples (glucose testing only), a low control and a high control for glucose must be run, along with the N control and P control. The urine low and high controls can be placed in the run where ever it is convenient.

Enter control results on excel quality control chart located on the T:drive.
(T:/vitreous/qualitycontrol/2014)

SAMPLE PREPARATION

1. Obtain a Vitreous Chemistry worklist from laboratory dataease program. (Urine requests also appear on this list)

2. Retrieve samples from refrigerator or freezer area of the laboratory. If sample is frozen, wait until it has thawed out completely.
3. Label biocups. Indicate year, toxicology number, specimen type, date and analyst's initial.
4. Vortex samples.
5. Handling one specimen at a time, pipet a **minimum** of 600 uL into a biocup. Close the cap on the biocup.
6. Centrifuge samples at 1800 rpm for 5 minutes.
7. Carefully remove samples from centrifuge.
8. Samples are ready for analysis. If analysis is delayed, place samples in the refrigerator.

NOTE: Biocups from SHORT SAMPLES (original vitreous or urine volume less than 1.5 mL) MUST be saved until the case has been closed.

DO NOT use up the entire original specimen. If dilutions are needed, consult a supervisor before proceeding.

NOTE: Biocups must be stored for a minimum of two weeks. If feasible, they should be retained until the case is closed (check the Main Form in Dataease for a FINAL date).

INSTRUMENT SEQUENCE

1. From the main screen menu, select the worklist icon.
2. Select "**Patient**" tab. Fill in the "**PID, Last Name and First Name**" fields with the Toxicology Laboratory number (Example: 14-0050). Enter any relevant information in the comment field. **Save after each entry.**
3. Go to the "**Selection**" tab. Select position # 1 on the left hand side of the worklist. Under "**Type**" select normal. Under "**Cup**" select biocup. Under "**Sample**" select common. Leave the "Range" field as Human Auto. Under "**PID**" select the Toxicology Laboratory number that will correspond to that position. Check off requested tests from the menu below. **Click save.**
4. The cursor will move to the next available position (#2). Repeat Step 3 until you have filled all positions from 1 to 13.. In position # 14, run N control. In position # 15, run P control..

NOTE: Only 15 positions are available for one run including controls. (e.g. If 15 samples are going to be run, then positions 14 and 15 are designated for N and P controls). Additional samples will be run in a separate run along with N and P controls. Adjust accordingly if fewer samples are run.

5. To enter sample information for the N control, go to "**Type**" and select control. Under "**Cup**" select biocup and under "**Control**" select N control. Check off requested tests from the menu below. **Click save.**

6. Repeat step # 5 for the P control.
7. Go to the bottom left of the screen and click on the arrow. A summary of the worklist will appear. Print the page by holding print button for **two** seconds.
8. Load samples in the sample tray according to the worklist. Make sure that each position correctly corresponds to the case listed on the worklist.

NOTE: Results that are below the analyzer range, short samples and “ISE waiting” will be flagged for a repeat analysis by the instrument’s software.

At the end of the run, look up the error code. From the main screen menu, press the “**Flag/Alarm**” icon, a red triangle located on the tool bar. Look at the specific flag/alarm code and continue accordingly.

For a list of flag/alarm codes refer to User’s manual page 117-120. If the instrument was unable to complete a test, the test will remain on the worklist. After troubleshooting, start the instrument by pressing the start button. The instrument will analyze any unfinished tests for the run.

DILUTIONS

1. Each test has a lower and upper analyzer range. A dilution is required if test results are above the analyzer range. Please refer to table below.

<i>Cl⁻</i> (mmol/L)	<i>K⁺</i> (mmol/L)	<i>Na⁺</i> (mmol/L)	<i>GLU</i> (mg/dL)	<i>BUN</i> (mg/dL)	<i>Creat</i> (mg/dL)
65 - 140	1 - 11	95 - 215	5 - 900	2 - 98	0.10 - 18

Start with a 1:2 or 1+1 dilution. Use the sample remaining in the biocup.

If more sample is needed, retrieve the original sample from refrigerator. Using an automatic pipet, aliquot needed amount into a 12 x 75 mm glass tube. Add the necessary amount of water to achieve the desired dilution.

EXAMPLE: 1+1 Dilution (1:2)

Pipet 400 µL vitreous humor or urine, then add 400 µL of water. Vortex the mixture. Label a biocup with case number, specimen type, dilution, date and analyst’s initials. Aliquot into a labeled biocup.

Set up a new sequence and include the dilution factor as part of the patient ID. Run diluted samples with appropriate controls following the **Instrument Sequence SOP** steps. Repeat with larger dilutions, as necessary, until results fall within the analyzer range. Multiply the final concentration by the dilution factor. (e.g., 1:2 or 1+1 = multiply end result by 2, 1:3 or 1+2 = multiply end result by 3) Dilutions must be noted on reports.

DATA REVIEW

1. Review data results for N and P control. Refer to acceptance criteria section of SOP.

2. Review data results for each case. Those that are within the analyzer range are reported according to the printed results. Calculate final concentration for diluted samples by multiplying the corresponding dilution factor with appropriate notation on the printout.

ACCEPTANCE CRITERIA

Results for the calibrator, and the N and P controls **must** fall within the established range as listed on lot specific vitreous chemistries calibrator and controls sheet provided by the manufacturer. Each if results are outside the established manufacturer's range or there is a flag, do not accept the run. The same applies to calibrators and controls purchased from a different manufacturer. (e.g. Pointe Scientific). On the print out for the N and P controls, affix a N control and P control label indicating the target mean and established range. On the label, write in the lot number for each control.

REPORT FORMS

1. Make copies of the sequence list and controls. (N control, P control, Low Urine control and High Urine control)
2. Affix a copy of the above to each case print out.

REPORTING

1. Results within the analyzer range are reported as follows:
 1. Chloride, potassium and sodium are reported in mmol/L
 - a. Chloride and sodium are reported with no decimal place.
 - b. Potassium is reported to one decimal place
 2. Glucose, creatinine and urea nitrogen are reported in mg/dL.
 - a. Glucose and urea nitrogen are reported with no decimal place.
 - b. Creatinine is reported to one decimal place.

NOTE: *Truncate decimal places to achieve the proper number of digits.*

It is possible that results may be affected by the viscosity of the sample, producing results that are physiologically questionable. This usually is noticeable as sodium (Na^+) or chloride (Cl^-) concentrations lower than 110 for the former and 100 for the latter. When this occurs, reschedule ALL tests requested using a 1:2 dilution to start. Re-evaluate when done, and use higher dilutions as needed.

2. Results with concentrations lower than the instrument linearity will be reported as less than (<) according to the following table:

Cl^- (mmol/L)	K^+ (mmol/L)	Na^+ (mmol/L)	GLU (mg/dL)	BUN (mg/dL)	Creat (mg/dL)
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<65	<1	<95	<20	<1	<0.1
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INSTRUMENT SHUTDOWN

At the end of the day the instrument will have to be put in sleep mode. From the main screen menu, select the “shutdown icon” on top of the tool bar. Select sleep mode from the pop up window. Refer to User’s manual page 15.

MAINTENANCE

1. **Daily procedures:** If more than 20 tests are performed per day, ISE electrode cleaning is necessary.
 - a. Open main cover. Open sample and reagent container unit cover.
 - b. Pour 2 mL of Horiba Etching solution into a 10x 75 mm glass tube. Place the etching tube on position # 14 in the sample tray. Close the SRCU cover. Close the main cover.
 - c. On the main screen menu, from the “Selection” tab select position 14. From the “Type” drop down menu, select ISE Cleaning 2. Click save.
 - d. Return to the main screen menu, select “Services” and then select “Customer Services”. From the “Sequence” tab, select ISE Cleaning 2. The instrument will start the ISE cleaning automatically. This will take 5 minutes. Wait until is the main menu screen says “Ready” to remove the etching tube from the sample tray.
 - e. Use a paper towel, wipe any condensation from the inside of the refrigerated compartment.
2. **Weekly procedures:** If more than 20 tests are performed per day, ISE electrodes cleaning is necessary with a Deproteinizer solution.
 - a. From the main screen menu, go to inventory to make sure there is enough deproteinizer on the reagent tray.
 - b. If there is not enough deproteinizer, then a new deproteinizer cassette must be loaded. Open main cover. Open SRCU cover. Remove sample and reagent tray. Load the new deproteinizer cassette. Place sample and reagent tray back in the refrigerated compartment of the instrument. Close the SRCU and main covers.
 - c. Select the inventory icon and perform a barcode scan.
 - d. On the left side of the instrument, open the ISE module and remove the Cl^- electrode and replace with a “dummy” electrode. Close the ISE module.
 - e. Go to shutdown icon, select sleep mode, select the On button, select the S button and press OK to start the wash. It will take 20 minutes to complete.

NOTE: This process should be done at the end of the day

- f. Before the Cl^- electrode is placed back in the ISE module, clean it with the Cl^- cleaning kit.
- g. Perform activation with human serum after cleaning ISE with deproteinizer. Aliquot 600 uL of human serum (UTAK). Place in sample tray position# 1. Go to the worklist icon, select

position # 1. Select ISE activation from the sample type dropdown menu. Go back to the main screen. Select services icon, select toolman and select ISE activation. This will start the ISE activation automatically. Once it finishes, calibrate the instrument for ISE and run controls.

- h. Probe cleaning – Power off the instrument. Lift up the probe unit by hand. Wipe the probe gently from **top to bottom** with gauze moistened with ethanol. Repeat above step using distilled water. Dry the probe with a soft cloth. Return the probe arm to the wash tower position.

NOTE: NEVER wipe the probe from bottom to top, it will damage the needle

3. **Bi-weekly procedures:** Perform tubing and external tanks decontamination.
 - a. Empty the water tank and the waste tank. Add 500 mL of 10% bleach solution to the water tank. Add 2000 mL distilled or reagent grade to water tank. Replace the respective tubing.
 - b. From the “Sequence” tab press tubing wash button. Tubing is filled with washing solution and a 10 minute incubation is performed for effective decontamination. When decontamination is completed, a pop-up window is displayed.
 - c. Empty the water tank and rinse with distilled water. Fill water tank with distilled water. Press OK to wash the tubing with distilled water. After process, discard any distilled water remaining in water tank.
 - d. Fill the water tank with distilled water. Prime the instrument to fill with distilled water. Type 6 for the number of primes. Press the prime button.
 - e. If necessary, **every 6 months**, change the filter located on the tubing of the water tank. Follow the arrows on the tubing and filter. Prime instrument after changing the water filter.

4. **Monthly procedures:**

- a. Replace the **Standard 1** and **Reference** solutions.
- b. Refer to User’s manual page 102. Once solutions have been replaced, run a precision test (**T1**).
- c. Remove the **SRCU** cover. The cassette has 2 main compartments. One side is a 30 mL compartment and the other one is a 10 mL compartment. Fill the 30 mL compartment of the cassette with 25 mL distilled water.
- d. Load the cassette on the reagent tray, position # 8.
- e. Pipet 500 uL of the **Qualitest** solution and 500 uL of distilled water onto a 10 x 75 mm culture tube. Mix well. Transfer to a biocup. Place the biocup on position 1 of the sample and reagent tray. Close the SRCU and main cover.
- f. Press barcode scan from the reagent tray screen. In the main screen menu, go to “**Order**” selection tab, and register the T1 test solution. Select the T1 test sample cup position. Select replicate under “**Type**”. Select biocup under “**Cup**”. Select 15 from the “**Number**” dropdown list. Choose the T1 test by checking the corresponding box. **Click save**.
- g. Press the start button for more than **two** seconds. When all the tests have been performed (15), use the results to calculate the mean and the CV using the instrument’s software. The

expected CV is $\leq 1.8\%$. If expected CV is not obtained, please refer to the Troubleshooting chapter of the User's manual.

5. **As needed basis:** Replace Chloride ISE electrode (4 months); Potassium ISE electrode (6 months); Sodium ISE electrode (1 year); Reference ISE electrodes (1 year), Replace Lamp as needed.
6. Record any maintenance performed on Pentra C200 instrument in maintenance log book.

TROUBLESHOOTING (FLAGS AND ALARMS)

Please refer to User's manual page 177 to look up a specific flag/alarm code error, description and error cause. Troubleshoot the specific issue.

1. Sample shortage or sample not being detected – open the SRCU, check the sample number that is causing the error. Make sure there are no bubbles in the sample. If a short sample, wrap parafilm around the biocup to create some resistance against the sample holder to increase the height of the biocup by a few millimeters without interfering with the SRCU cover.
2. IE1, IE2, IE3 (ISE measurement error) – open the SRCU, check the volume of the sample that is causing the error. Add more sample to the biocup. Centrifuge and analyze for ISE on the next run.
3. R1S and R2S (Reagent shortage) – from the main menu, see if any circles that are red . If so, click on the cassette icon. Go to inventory and check all of the reagents listed there. Those in red are completely empty and need to be changed in order to continue analysis. Those in orange are running low. Keep in mind those reagents that will need to be changed in the near future. Change the reagents needed and reset the volume.
4. CTO (Calibration expired) – run a new calibration for ISE, glucose, urea and creatinine. Remember, **ISE calibration is good for 8 hours**. The stability for glucose, urea and creatinine calibration is significantly longer. To find calibration information, on the main menu, click the inner or outer circle of each reagent. A pop up window will appear. Name of the reagent, volume, # tests remaining, lot expiration date and stability will be displayed.
5. Q (Control error) - executed the measurement with a control error. Run a new calibration and control.

Contact Technical Services at 1 (888) 903-5001 for an issue you are not familiar with. Be ready to provide the instrument's S/N which is S/N 112C20509.

Log the reason for the call into the instrument maintenance book. Once you hear from Horiba Technical support, explain to them the nature of the call and record their advise/instructions in the instrument maintenance log book..

NOTES

1. Please make sure that calibrators, controls and samples are **free of bubbles** upon loading into sample tray.

2. The time clock icon located on the bottom left of the main screen menu only displays the time it will take for analysis of unknowns.
3. Horiba **MCal** can be used for **7 days** after reconstitution. N and P controls can be used for **7 days** after reconstitution. **Store refrigerated at 2-8° C.**
4. Requested tests are indicated on the Vitreous Chemistry work list.

Example

<u>TOX #</u>	<u>K+</u>	<u>Na+</u>	<u>Cl-</u>	<u>Glu</u>	<u>BUN</u>	<u>Creat</u>
<u>13-0001</u>	<u>x</u>	<u>x</u>		<u>x</u>	<u>x</u>	<u>x</u>

A volume of 1.5 mL of sample is more than enough to perform all tests.

NOTE: If the vitreous sample volume is **1 mL or less** review the case folder. If other tests are scheduled in vitreous (e.g. for volatiles and/or drugs), do **NOT** continue with the analysis and consult a supervisor.

REFERENCE

Pentra C200 User's Manual, Horiba Medical Company, Irvine, California, 2010.

*The above reference is available on OCME T drive under T: /chemistry/PentraC200usermanual

There is a printed copy on a binder next to the instrument for your reference.

REVISION HISTORY

Ver 02.27.2014

1. Revision history implemented.