| Wake Forest Baptist Medical Center | Intraoperative PTH testing on the Roche Cobas e411<br><br>CCL-031 | Dept: 324318 | Critical Care Labs |
|---|---|---|---|
| | | Effective Date: | Dec 2018 |
| | | Revised Date: | March 2019 |
| | | Contact: | Ann Shoffner |
| Name & Title: Gregory Pomper, MD<br>CLIA Laboratory Director | | Date: | 3/8/19 |
| Signature: G Pom | | | |

1) **General Procedure Statement:**
   a. **Purpose:** Immunoassay for the in vitro quantitative determination of intact parathyroid hormone in human plasma. This assay is used intraoperatively.
   b. **Principle:** The Cobas e411 uses ECL (Electrochemiluminescence) technology – where a light reaction is initiated by electrical stimulation of molecules. This ECL quality is integrated with conventional Ag and Ab reactions. When the reaction takes place, light is emitted and read. Duration of assay = 9 minutes.
   c. **Responsible Department/Party/Parties:**
      i. Procedure owner: Starr Hill/Jeff Johnson/Travis Cline/Michelle Brown/Ann Shoffner
      ii. Procedure: Critical Care Lab (CCL) Staff
      iii. Supervision: Ann Shoffner
      iv. Implementation: Ann Shoffner and Critical Care Lab (CCL) Staff

2) **PPE Requirements:** Lab Coat. Gloves. Eye protection when preparing SysWash.

3) **Procedure:**
   A. **Instrument Temperature and Humidity Requirements:**

| | |
|---|---|
| Temperature - | Operation: 18 to 32°C |
| | Storage and transport: -20 to 75°C |
| Humidity - | Operation: 20 – 80% (non-condensing) |
| | Storage and transport: 5 to 95% (non-condensing, <80% average per year) |
| Altitude - | Operation: up to 2000m |

   B. **Sample Requirements:**
      1. **Acceptable Sample types** –K2 EDTA plasma, K3 EDTA plasma and Serum are acceptable samples for analysis. EDTA plasma is preferred due to longer stability and faster processing.
      2. **Sample Labeling** – For sample labeling requirements and discrepancy resolution, refer to CCL-032 General Specimen Information.
      3. **Sample Handling** – Centrifuge samples prior to testing. Ensure that samples, QC and Calibrators are at Room Temperature (20 – 25 C) prior to testing.
      4. **Stability Limits:**
         Serum: Stable for 8 hours at 15-25 °C, 2 days at 2-8 °C, 6 months at -20 °C.
         Plasma: Stable for 2 days at 15-25 °C, 3 days at 2-8 °C, 6 months at -20 °C.
         Due to possible evaporation effects, samples, calibrators and controls on board the instrument should be analyzed within 2 hours.
      5. **Sample Volume:** 50uL
      6. **Sample Retention:** Store EDTA plasma samples for 3 days at 2-8 °C
      7. **Sample rejection criteria:**
         - Samples exceeding stability limits for the specimen type will be rejected.
         - Hemolysis - this assay is affected by hemolysis $\geq$ 0.25 g/dL (moderate hemolysis).
         - Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

C. **Reagents and Supplies:**

1. **PTH STAT reagent Kit** (part # 04892470 160) - The reagents in the kit have been assembled into a ready-for-use unit that cannot be separated. All information required for correct operation is read in from the reagent barcode.

| Stability: | |
|---|---|
| Unopened at 2-8 C | up to stated expiration date |
| After opening at 2-8 C | 12 weeks |
| On the analyzer | 8 weeks |

****Important:**

- **The PTH STAT Reagent must be removed from its cardboard box and brought to room temperature before loading on the analyzer (45 minutes – 1 hour).**
- **Reagent packs must be stored upright, not positioned on their side.**

2. **Bio-Rad Liquichek Specialty Immunoassay Control Levels 1, 2, 3**
   - Product does not require reconstitution.
   - Product is stable until the expiration date when stored unopened at -20 to -70°C.
   - Once thawed, opened and stored tightly capped at 2 to 8°C, this product stable for 7 days.
   - Once thawed, opened and stored in tightly capped aliquot vials at -20 to -70°C, this product is stable for 30 days.
3. **PTH STAT CalSet** (part # 08243930190) PTH STAT Cal 1 and PTH STAT Cal 2. (see Calibration section for more details)
4. **SysWash** (part # 11930346 122) – Increases the rinsing efficiency between pipetting steps. 35mL of SysWash is mixed with 3000 mL of Deionized water and placed on the instrument. SysWash is stabile on board the analyzer for 2 weeks. Label the on board instrument container with the date the SysWash was made and the 2 week expiration date. In addition to the date prepared and expiration dates, the on board instrument container is considered a secondary container and should have a label that contains the content, quantity, concentration or titer and the storage requirements.
5. **ProCell** (part # 11662988 122) - system buffer. Stable on the analyzer 4 weeks not to exceed 72 hours open total. The ProCell should be closed after use. The ProCell should be changed weekly.
6. **CleanCell** (part # 11662970 122) - measuring cell cleaning solution. Stable on the analyzer 4 weeks not to exceed 72 hours open total. The CleanCell should be closed after use. The CleanCell should be changed weekly. CleanCell is also used for the bi-weekly liquid flow cell cleaning.
7. **SysClean** (part # 11298500160) – An alkaline cleaning solution with antimicrobial properties used for flow cell cleaning.
8. **Deionized H20** – Obtained from Blood Bank
9. **PTH CalCheck5** (part # 5918103160) - an assayed control for use in calibration verification and for use in the verification of the established assay range.
10. **PreciControl Varia** (part # 05618860160) – This is Roche's brand of quality control material. It is kept on hand for use in troubleshooting. If needed, reconstitute with 3mL of deionized H2O delivered from a Class A volumetric pipette. Note: calibrators and controls should be prepared separately.

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**D. Powering On, Sample Reception Mode, Sleep Mode, Shutdown:**

To maintain the temperature of the reagents, leave the analyzer circuit breakers switched on.

1. **Powering ON the system from Sleep Mode:**
    1. Toggle the switch on the front bottom left of the analyzer to the ON position. The system shuts down, reboots and comes back up,
    2. The system goes thru *Initialization*
    3. The system goes to *Stand-By*
    4. Log onto the system. Operator ID: admin (lowercase) / Password: admin (lowercase)
2. **Powering ON the system from Shut Down:**
    1. Switch on the printer
    2. If the analyzer circuit breakers are off, then switch them on
    3. If the rack circuit breakers are off, then switch it on
    4. Toggle the switch on the front bottom left of the analyzer to the ON position.
    5. The system goes thru *Initialization*
    6. The system goes to *Stand-By*.
    7. Log onto the system. Operator ID: admin (lowercase) / Password: admin (lowercase)
3. **Sample Reception Mode:** When Sample reception mode is active the system remains in "Operation" mode. Being in "Operation" mode allows you to continue running samples without going through the initialization process. Sample reception mode can be set for 1, 2, 4 or 8 hours. Reset the time as needed to better accommodate the daily OR schedule. On days with no OR cases the sample reception mode can be deactivated. The Sample Reception Mode is activated at the START screen.

    **NOTE:** Should the instrument be in STANDBY when you anticipate a patient sample you can activate the Sample reception mode before your first patient sample arrives by: Placing the "Stop" tube in position 1 and starting the instrument. This puts the instrument through initialization without actually running a patient sample.
4. **End of Day Activities/Sleep Mode:**
    1. *Finalization maintenance*: When the system is in Sample Reception mode it automatically performs *Finalization maintenance* before it goes to *Stand-By*. If it is not in Sample Reception mode or a sample has not been run on the instrument, you will need to manually initiate a *Finalization*. To do this, select the *Maintenance* function from the System Overview screen. Select option 7 - *Finalization Maintenance* then press START. **Finalization Shortcut:** *System Overview → Maintenance → Finalization Maintenance → START*
    2. At end of day, close the lids on the ProCell and CleanCell.
    3. Put the instrument into **Sleep Mode** by toggling the switch on the front bottom left of the analyzer to the OFF position. In Sleep Mode, power consumption is reduced. Power is still supplied to the reagent rotor and system reagent compartments to keep them cool.
5. **Shutdown:** Under normal circumstances, you do not need to shut down the system. The temperature of the reagents will not be maintained when the system is shut down.

    To shut down the system:
    1. Choose the global *Logoff* button
    2. From the *Logoff* dialog box, choose the *Shutdown* button
    3. Choose *OK*
    4. Switch off the circuit breaker on the right side of the analyzer
    5. Switch off the circuit breaker on the rack feeder

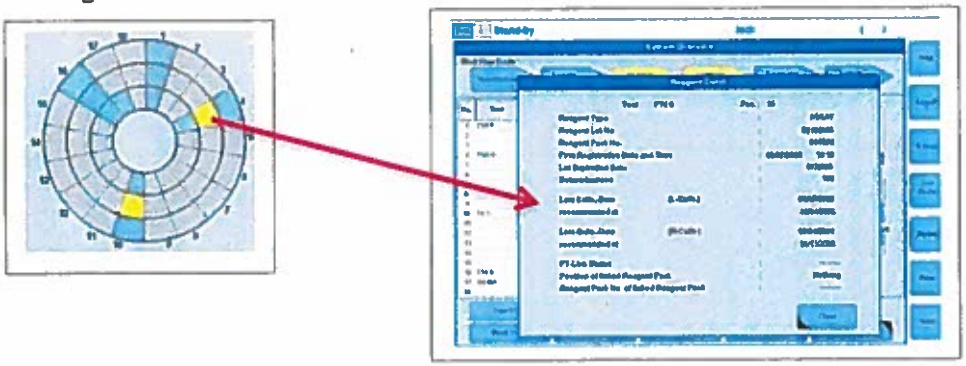
**E. Instrument Maintenance:**

Perform all routine maintenance for the instrument according to the manufacturer's instructions found in the *Cobas e411 Maintenance Guide*. Document all routine an unscheduled maintenance on *CCL-F094 Cobas e411 Maintenance Log* found in the *Current Year's Maintenance, QC and Patient Logs.*

**Daily Maintenance:**

- Clean the Sample/Rgt probe w/DiH2O and gauze (pg 212) probe on the left
- Check condensation inside reagent and ProCell (PC)/CleanCell (CC) compartments and remove if necessary (pg 215-218)
- Open PC/CC lids. Check open/expiration dates and replace if needed. Once opened on the system, they are good for 72 hours.
- Check and remove empty tip/cup tray(s)
- Check system water volume (change bi-weekly or as needed)
- Check and empty liquid waste if necessary
- Turn instrument on
- Check system alarms (pg 99-100)
- Check the calibration status and reagent volume.
  After the initial calibration is performed for a lot of reagent, the analyzer does not readily flag you when it is time to update either the lot calibration or the reagent pack calibration. To determine if a calibration is due, perform a Reagent Scan.

- Following the Reagent Scan, touch the reagent rotor segment for the pack to display the *Reagent Details* window. Check the lot calibration "recommended at" date. Also check the reagent volume. If there are less than <30 determinations (tests remaining) load a new reagent pack (pg 105) and perform a reagent scan.



- Run daily QC (pg 137-138)

**Weekly Maintenance:**

- Replace PC/CC. They go in station 1. New bottles must be on instrument at least 15 minutes or you will get a temperature error. We don't load PC/CC in station 2.
- Perform system shutdown (pg 202-203). Go to STDBY. Click Logoff button. Cut off circuit breaker right side. Leave off 30 seconds and turn back on
- Perform Reagent calibration (Chapter 7) – See Section F Calibration
- Clean incubator & aspiration station (pg 221-223)
- Clean sipper probe (pg 219-221)

**Every 2 Weeks Maintenance:**

- Liquid flow cell cleaning (Cleaning the sipper probe system) -pg 223-228.
- **Important**: Remove the PC/CC from position 1 and place in position 2 – the SysClean adapter goes in Position 1.
- Replace system water and as needed. Fill DiH2o to the top line, add 35mL of SysWash. Once reconstituted it is good for 2 weeks.
- Clean rinse stations (pg 229-230)

**Monthly Maintenance:**

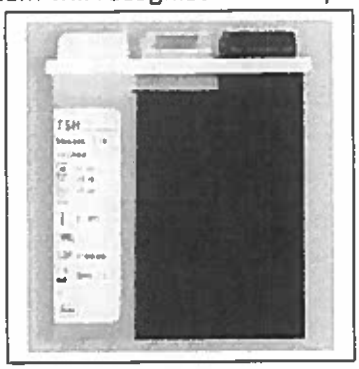
- Replace pinch valve tubing (pg 231-237)
- Export data to USB drive and give to Lab Manager to save in ICU_OR/Lab Cobas e411/Export data

**As Needed Maintenance:**

- Clean system water container (pg 237-240)
- Clean liquid waste container (pg 240-242)
- Clean PC/CC compartment (pg 245-247)
- Change solid waste tray liner (pg 252-255)
- Clean micro-bead mixer (pg 244-245)
- Clean reagent compartment (pg 247-251)
- Clean sample rack and analyzer surfaces (pg 251-252)

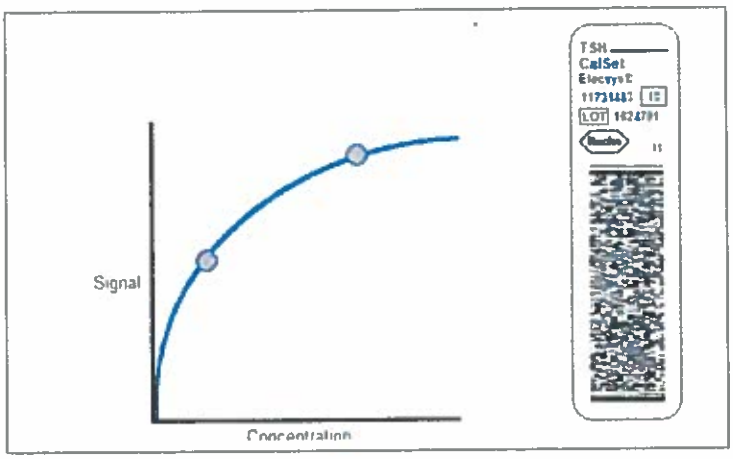
**F. Loading a new reagent cartridge:** Refer to section J. if the reagent pack is a new lot number.

1. Allow reagents to warm to room temp for 45 minutes before loading them onto the instrument. CHECK FOR BUBBLES!
2. Reagents can be added only while in Stand-by or sleep mode.
3. Open the lids slightly on the reagent pack.
4. Write the open date, expiration date (8 weeks from open date) and your initials on the reagent pack
4. Open the reagent compartment lid on the analyzer
5. Install the reagent in any of the available reagent slots (the compartment has notches so it will only install the correct way)
4. From the System Overview screen, perform a *Reagent Scan* after reagents have been added so the system will recognize the newly loaded reagents.



**G. Instrument Calibration:**

The utilization of a lot-specific master calibration curve eliminates the need for a 5 or 6 point calibration on the analyzer. A 2-pt calibration is all that is required. The values of the CalSet calibrators are determined from the master calibration curve and are then encoded on the CalSet 2-D barcode card. CalSet calibrators are used to update two of the four Rodbard curve defining parameters. This allows the curve encoded on the reagent pack 2-D barcode to be adjusted to our analyzer.




1. **Calibration frequency:** Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).
   Calibration is recommended as follows:
   - Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).
   - After 12 weeks when using the same reagent lot
   - After 7 days (when using the same reagent kit on the analyzer)
   - As required: e.g. quality control findings outside the defined limits or if QC material reflect an unusual trend or shift.
   - After major repair or change of a critical instrument component
   - If calibration fails, repeat the calibration
2. **Calibrator Preparation/Handling:** Calibrators are prepared using Class A Volumetric pipettes only. Add exactly 1.0 ml of distilled or deionized water from a Class A volumetric pipette to the contents of the calibrator bottle. Allow bottle to stand closed for 15 minutes. After 15 minutes, mix carefully, avoiding foam formation. Transfer the reconstituted calibrators into the supplied empty labeled snap-cap bottles. Ensure the calibrators are at room temperature (20-25°C) before measurement.
3. **Calibrator stability:** The reconstituted calibrators should only be left on the analyzer during calibration. After use, close the bottles as soon as possible. Calibrators should not be exposed to air for more than 5 hours total. Track the number of times the calibrator is used on the instrument by marking a line on the top of the calibrator when the calibrator is used. Reconstituted calibrators should be stored upright at 2-8°C.

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4. **Performing a Calibration:** Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

    1. If the Calibrator is a **new lot number**, scan barcode card for the new lot number of calibrator as follows: From the System Overview screen, select *Calibration*. Select *Install* at the top of the screen. Insert the barcoded calibrator card (face backwards) into the barcode reader slot underneath the "boom". Select *Scan BC Card* on the computer screen.
       **New Lot Calibrator shortcut:** *System Overview → Calibration → Install →* insert the calibrator card *→ Scan BC Card*
    2. Load Cal1 and Cal2 next to each other.
    3. Open the lids on the calibrators.
    4. Assign the calibrators a position: From the System Overview screen select *Calibration.* Select the *Calibrator* tab. Select *Position Assignment*. Select the calibrator you want to assign and the position you want it to occupy. Select *Assign*.
       **Assign Calibrator Position Shortcut:** *System Overview → Calibration → Calibrator → Position assignment →* select the calibrator position *→ Assign → Start → Start*
    5. After you have finished assigning the calibrator positions, Hit OK
    6. Place the calibrators on the wheel according to the positions you assigned
    7. Select START and START
    8. After calibration is performed un-assign the calibrators from its positions:
       **Un-assigning the Calibrators Shortcut:** *System Overview → Calibration → Calibrator → Position assignment → Remove → OK.*

**H. Quality Control:**

1. **Frequency:** 3 Levels of BioRad Liquichek QC (1, 2, 3) are tested M-F (excluding holidays) on 1st shift. QC is performed regardless of a scheduled case. Controls are run prior to reporting patient results after reagent change, major maintenance or change of critical instrument components.

2. **Running a QC sample**:
   Allow QC to warm to room temperature before analysis
    1. From the System Overview screen, select the *QC* function
    2. Select the *Control* tab
    3. Select *Position Assignment*
    4. Select the QC you want to assign and the position you want to assign it to
    5. After you have finished programming, press the *OK* button
    6. Press *START* and *START*
    7. Clear the QC programming once the QC process is complete (See Section 8 below)

   **QC Shortcut:** *System Overview → QC → Control → Position Assignment→* select the QC and its position *→ OK → START → START*

3. **Running QC on a Standby reagent pack:**
    1. From the System Overview screen, select the *QC* function
    2. Press "QC status"
    2. Press "Stand by Bottle QC"
    3. Select the newly loaded reagent cartridge (a check mark now appears in the selection column). Press "OK"
    4. Program QC positions as in running QC samples.
    5. Press *START* and *START*

   **Standby QC Shortcut:** *System Overview → QC → QC Status → Stand by Bottle QC →* select the reagent cartridge *→ OK →* Program QC samples *→ START → START*

4. **Interpretation of QC results:** Control results are reviewed and deemed acceptable before patient results are reported. The Critical Care Labs have adopted the policy of repeating all out of range QC values. One may not report patient data until all QC failures are resolved and the test method is demonstrated to be "in control".

5. **If QC fails:**
    1. Repeat the QC level.
    2. If QC continues to be out, review the QC data for the past 30 days in Beaker looking for trends and or shifts. Refer to the Beaker Quick Reference Guide for how to review QC results in Beaker.
    3. Calibrate.

6. **Documentation of QC results:**
    a. Order and result the QC in Beaker using the Quality Control function.
        QC Test = WC QC BG OR LAB – CHEM MANUAL
        QC Material = ORLAB IOPTH CHEM 1, ORLAB IOPTH CHEM 2, ORLAB IOPTH CHEM 3.
        For more detailed instructions, refer to *Ordering and Resulting QC* in the *Beaker Training Guide*.
    b. File the QC instrument reports in the front of the binder labeled *Roche Cobas e411 Current Month QC Printouts.*

7. **If you have a patient sample to test post running your QC:** If you need to run a patient sample post QC before the Cobas has gone into *Stand-By*, you can just replace the stop tube with your 1st sample. In this case the system has not gone into standby and will continue to process samples in order around the wheel.

8. **Clear QC assignments - If you do not have a patient sample to run post your QC:** After QC is performed and all values are within acceptable limits, un-assign the QC from its positions. If the Cobas status displays *Stand-By*, it automatically goes to cup 1 to look for a programmed sample. If QC has been previously assigned to cups 1, 2 and 3, the Cobas will tell you that there is already a sample assigned to those cups. This has the potential to create confusion when programming patient samples. Remove non Roche QC assignments for those cups by performing the following steps:
    **Clearing QC Shortcut:** *QC → Control →* select QC *→ Position Assignment → Select Position → Remove→ OK*

9. **New Lot of BioRad QC:** For each new lot of control material, the manufacturer's supplied range is verified and a valid range for our lab established before placing the new lot into use. New lots of BioRad Liquichek are run in parallel with the existing QC lot (20 days) in order to establish the QC range and to verify that it is within the manufacturer's QC limits.

I. **Patient Sample Testing:**

**NOTE:** Should the instrument be in STANDBY when you anticipate or receive a patient sample you can activate the Sample reception mode before your first patient sample arrives by: Placing the "Stop" tube in position 1 and starting the instrument. This puts the instrument through initialization without actually running a patient sample.

1. **Receiving/Ordering Patient Testing in Beaker:**
    Samples should come to the OR Lab ordered with a Beaker barcode label affixed to the sample. Scan the barcode label in Beaker's Receiving Function to receive the sample into our laboratory. For assistance, refer to *Receiving a sample into the Lab* in the *Beaker Training Guide.* If the sample is not ordered in Beaker, manually place an order in Beaker - Manage Orders using the test code LAB3020. Alternatively, you can search using PTH. For assistance, refer to *Manual Ordering via Manage Orders and Order Inquiry* in the *Beaker Quick Reference Guide.*

2. **Sample Preparation:**
   Spin the sample and transfer approx. 0.5 mL to a sample cup. Sample cups used in IOPTH testing should be labeled with a Beaker aliquot label or identified using either the MR# or the digits following the BG in the Beaker specimen ID (ex 18W-326BG**0123.** The patient's last name alone is not an acceptable identifier.

3. **Load your sample onto the sample wheel:**
   - If the Cobas status displays "STDBY" (Stand By mode), it automatically goes to cup 1 to look for a programmed sample.
   - If the Cobas displays "S.Stop", replace the stop tube with your sample. In this case the system has not gone into standby and will continue to process samples in order around the wheel.
   - When you have put your last sample in the rack place the stop tube in the next empty position

4. **Sample Programming:** Program a patient sample as follows:
   1. Go to workplace
   2. Test selection
   3. New sequence
   4. Enter Position number (the cup number for your sample)
   5. Enter the patient's MRN or the digits following the BG in the Beaker specimen ID for sample ID and a brief specimen description
   6. Select IPTH for the test
   7. SAVE

   The analyzer will auto advance to the next position for another sample.

   **2 Very Important STEPS:**
   - **The Boom must be down before you press START!**
   - **The ProCell and CleanCell bottles must be open!**

   8. Make sure the instrument is in S. Stop mode
   9. START and START

   **Sample Programming Shortcut:** *Workplace* → *Test Selection* → *New Sequence* → Enter position number → Select test → *Save* → *START* → *START*

5. **If you forget to lower the Boom:**
   1. Delete the Alarm
   2. Go to the Maintenance function and reset the system

6. **To add a sample(s):**
   1. Program samples into keyboard
   2. With Cobas in "S.Stop" move the stop tube and place samples on wheel and press *Start*. It will take a minute for the Cobas to recognize your entry.

7. **To delete a sample you have programmed:**
   The instrument must be in standby to delete a patient record.
   1. Go to workplace
   2. Data Review
   3. Find sequence number that you want to delete
   4. Highlight the patient you want to delete
   5. Select **DELETE RECORD.** You can only delete one record at a time

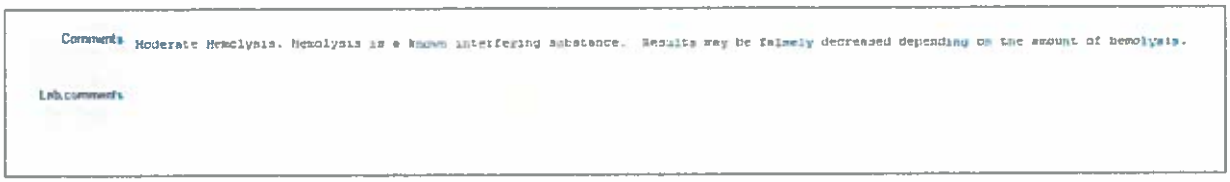
8. **To delete a sample after you have started the run:**
   1. Press STOP.
   2. When the analyzer goes into standby use the procedure above to delete the patient record.

**9. Interpretation of patient results:**
PTH has a reported half-life of 3-5 minutes. A significant drop in PTH levels after resection of the abnormal parathyroid gland or glands enables the surgeon to assess the completeness of resection and whether all hyper-functioning parathyroid tissue has been removed from the patient. A > 50 % reduction in PTH levels from the highest baseline is used as criteria for surgical success.

UOM: pg/mL
Reportable Range: 1.2 – 5,000 pg/mL
Values below 1.2 should be reported as <1.2.
Values greater than 5,000 should be reported as >5000.
Reference (Normal) Range: 15 – 65 pg/mL
Critical Values: None

**10. Reporting Patient Results:** Results should be manually entered in Beaker. Refer to *Manually Resulting in the Beaker Quick Reference Guide* for detailed instructions. Specimen description (Pre-excision, post-excision, 5 min, 10 min, etc) should be entered in the Comment section of the results screen if they were not entered when the order was placed.

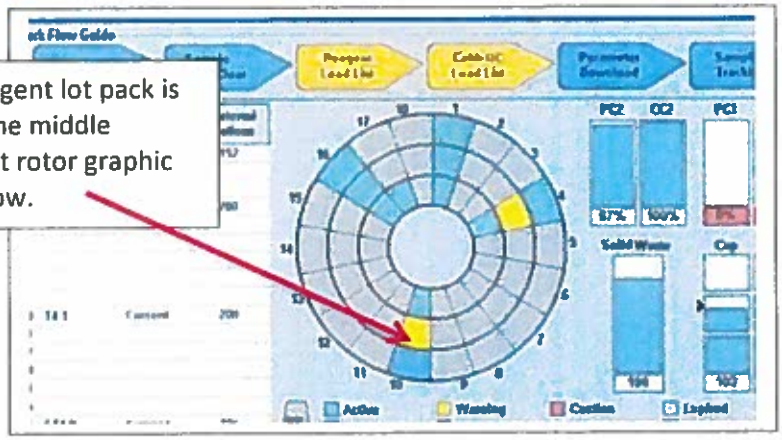
**Reporting Hemolysis:** This assay is affected by hemolysis ≥ 0.25 g/dL (moderate). There is a hemolysis smart text code unique to our IOPTH test. It is **HMOL** and it translates to "Hemolysis is a known interfering substance. Results may be falsely decreased depending on the amount of hemolysis." If the sample is hemolyzed, report the grade of hemolysis and the .HMOL code in Beaker's main Comments box under the result. You can use all smart texts: **.MODH** space **.HMOL**. Note: do not try to put the comments in the box to the far right of the result as that box is already populated with other information that reports with each result.




**11. Retention of patient printed results:** Patient result printouts from the Cobas e411 should be placed in the front of the notebook labeled Roche Cobas e411 Patient Result Printouts. These records will be kept for 2 years.

**J. New lot of reagent – lot to lot validation:**

1. Verify each new reagent pack that arrives. Place either a green "this lot # of PTH Rgt has been verified & is OK to use" or a pink "NEW LOT of PTH RGT Before use, compare old lot to new lot w/ pt samples & log"
2. Note: when a new reagent lot pack is loaded and scanned, the middle segment of the reagent rotor graphic for the pack turns yellow.



**New lot of reagent – lot to lot validation continued...**

3. In addition to QC, 3 patient samples spanning the low, mid and high range are run on both the old lot pack and the new lot pack. These samples are frozen at -20°C and are stable for upt to 6 months. Per Dr. Pomper, the values between the patient samples from the old lot to new lot should agree +/- 20%. Results are recorded on CCL-F096 PTH Rgt Lot to Lot Comparison log.

**K. AMR and Calibration Verification:**

Calibration verification involves the assaying of commercially prepared materials with known concentrations to verify that the calibration of an instrument, kit or test system has remained stable throughout the reportable range established for the laboratory. The reportable range of an assay is the range of values that the laboratory reports for that assay. The analytical measurement range (AMR) is the range of analyte values that a method can directly measure on the specimen without any dilution or concentration. Calibration verification verifies system performance in the clinically significant ranges, checking the upper and lower limits of the reportable range of patient results. Calibration Verification is not a requirement of the Cobas e immunoassay systems based on the manufacturer's recommendations. However, in instances where such a test procedure is required by certification agencies, or where the user wishes to document calibration verification, these CalCheck solutions provide an appropriate material for such testing. CAP defines Calibration Verification by referring to two distinct processes: 1) validation of the current method calibration and 2) validation of the assay range.

1. **Frequency:** Calibration verification is performed upon initial use of a system and every six months thereafter. If QC materials reflect an unusual trend or shift or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem. After major preventive maintenance, relocation, manufacturer stipulation or change of a critical instrument component.
2. **Materials:** Roche PTH CalCheck 5 contains 5 levels (approx. $\leq$ 1.20, 60, 2500, 4000, 5000 pg/mL). Each bottle, reconstituted to 1.0 mL.
3. **Target Values and ranges:** The target values and ranges were determined and evaluated by Roche. They were obtained using the Elecsys PTH assay reagents and analyzers available at the time of testing. The exact lot-specific target values and ranges are printed on the lot-specific value sheet. Results must be within the specified ranges. If results are out of range, then all test steps must be checked.
4. **Assay:**

   **a. Calibration verification only** - recommended levels are Check 2, 3 and 4.

   1. Using a Class A volumetric pipette, reconstitute each vial with exactly 1.0 mL of distilled or deionized water.
   2. Allow the bottles to stand closed for 15 minutes.
   3. Mlix gently by inversion.
   4. Stability reconstituted: 5 hours at 20-25°C
   5. Run the recommended levels of this CalCheck in duplicate on the cobas e411 analyzer. Program the samples as you would patient samples.
   6. Determine the average value for each level and compare it to the acceptable range listed in the electronically available value sheet. The average value should fall within the specified limits.
   7. If Check 2, Check 3 or Check 4 does not fall within the specified limits, repeat to exclude error in technique. If recovery is still outside the specified limits, contact Technical Support.

**b. Verification of the assay range only or verification of the assay range and calibration verification** - recommended levels are Check 1,2,3,4 and 5

1. Follow steps 1 – 6 above.
2. If Check 2 or Check 3 does not fall within the specified limits, repeat to exclude error in technique. If recovery is still outside the specified limits, contact Technical Support.
3. If Check 4 exceeds the assay range, mix equal parts of Check 3 and Check 4. Analyze the diluted sample in duplicate. If recovery is still above the assay range, contact Technical Support.
4. If Check 5 exceeds the assay range, Mix equal parts of Check 4 and Check 5. Analyze the diluted sample in duplicate. If recovery is still above the assay range, contact Technical Support.
5. If the diluted sample of Check 4 and Check 5 from the previous step still exceeds the assay range, mix equal parts of Check 3 and Check 5. Analyze the diluted sample in duplicate. If recovery is still above the assay range, contact Technical Support.

**L. Instrument Troubleshooting:**

**Service Hotline/Technical Information:**

Refer to the Troubleshooting section of the Cobas e411 analyzer Operator's Manual for detailed troubleshooting instructions. If normal troubleshooting efforts fail, contact Technical Support at 1-800-428-2336 (Instrument Serial # 65G6-13).

Document all troubleshooting actions on the instrument log sheet located in the *Current Year's Maintenance, QC and Patient Logs* notebook.

**Action if instrument is down:** Alert the appropriate patient care provider(s). The sample may be referred to the Core Lab for testing. However, there is a known bias that occurs between the 2 methods especially when our result is above 200 pg/mL. Our results are expected to be about 20-30% lower than the Core Lab. If we must refer samples to the Core Lab, it would be best to refer all the samples from the case so that results can be more accurately evaluated. If we are inoperable after the case has begun and our lab has resulted the first result, then the Core Lab would need to rerun the samples that we have previously run and our results would need to be corrected and replaced by those obtained the Core Lab.

**M. Limitations of Procedure:**

The assay is affected by hemolysis ≥ 0.25 g/dL (moderate). The assay is unaffected by icterus (bilirubin < 1112 µmol/L or < 65 mg/dL), lipemia (Intralipid < 1500 mg/dL), and biotin (< 205 nmol/L or < 50 ng/mL). Criterion: Recovery within ± 10 % of initial value. Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration. No interference was observed from rheumatoid factors up to a concentration of 1500 IU/mL. There is no high-dose hook effect at PTH concentrations of up to 17000 pg/mL (1802 pmol/L). In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found. In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

**N. Assay Process:**

- 1st incubation: 50uL of sample, a biotinylated monoclonal PTH-specific antibody and monoclonal specific antibody labeled with a ruthenium complex form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated micro-particles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the micro-particles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode or e-barcode.

**O. Complexity: Moderate per CLIA**

**P. Pipette Carryover: None per manufacturer**

**Q. Pipette Accuracy:** Per the manufacturer, for the end-user to verify accuracy and precision by gravimetric or photometric means would require the instrument to be run in a non-standard (and possibly unsafe) manner, and would indeed be "....not practical for the end-user laboratory..", as stated in the CAP wording. The performance checks carried out by your Roche Field Service Representative (FSR) during installation and preventive maintenance and troubleshooting, verify pipettor accuracy and precision.

4) **Related Procedures:**
CCL-002 Quality Control (QC) Plan
CCL-032 General Specimen Information

5) **Attachments:** none

6) **Related Forms:**
CCL-F094 Cobas e411 Maintenance Log

7) **References:**
Cobas e411 Maintenance Guide
Cobas e411 analyzer Operator's Manual
Ordering and Resulting QC in the Beaker Training Guide

8) **Related CAP Standards:** COM.30550, COM.30450

9) **Review/Revision/Implementation:**
- Review Cycle: All procedures must be reviewed at least every 2 years.
- Office of Record: Department of Pathology, Critical Care Laboratory

10) **Previous Revision Date(s):** 12/18

11) **Revised/Reviewed Dates and Signatures:**

Reviewed/Revision Date:_____ Signature:_____

Reviewed/Revision Date:_____ Signature:_____

Reviewed/Revision Date:_____ Signature:_____