PTH
INTACT PARATHYROID HORMONE
ADVIA Centaur XPT

By Chemiluminescence
LIS ORDER CODE = PTHWCA

I. Principle

The ADVIA Centaur Intact PTH assay is a two-site sandwich immunoassay using direct chemiluminescent technology, which uses constant amounts of two anti-human PTH antibodies in the Lite Reagent and an antihuman PTH antibody in the Solid Phase Reagent. The first antibody is a polyclonal goat anti-human PTH (N-terminal 1-34) antibody labeled with acridinium ester. The second antibody is a biotinylated polyclonal goat anti-human (39-84 region) antibody. Streptavidin in the Solid Phase is covalently coupled to paramagnetic latex particles. There is a direct relationship between the amount of PTH present in the patient sample and the amount of relative light units (RLUs) detected by the system.

II. Clinical Significance

Parathyroid hormone (PTH), produced by the parathyroid gland is the major circulating factor regulating extracellular calcium concentration. Abnormally low ionized calcium concentrations trigger the secretion of PTH. The PTH molecules bind to type 1 parathyroid hormone receptors in target tissues and initiate a sequence of reactions that result in an increase in extracellular calcium concentrations. PTH stimulates osteoclastic bone resorption resulting in the release of calcium from bone. PTH stimulates transcellular calcium reabsorption from the renal tubules and stimulates the kidney to produce 1,25-dihydroxyvitamin D which acts on the intestines to increase calcium reabsorption. In most clinical conditions, rising levels of extracellular calcium will suppress PTH secretion through a negative feedback mechanism.

Parathyroid hormone increases the rate of bone metabolism. Depending on the age of the patient, the bones involved, and the concentrations of the hormone in circulation over time, the effect on the bone can be either catabolic or anabolic. Consistently high concentrations of PTH generally have a catabolic effect and intermittent slightly elevated concentrations have an anabolic effect.

Quantification of circulating intact PTH assists in the differential diagnosis of hypercalcemia and hypocalcemia. In conjunction with the measurement of ionized calcium, intact PTH evaluations can be used to distinguish between patients with hyperparathyroidism, hypoparathyroidism, or hypercalcemia of malignancy. The diagnosis of primary hyperparathyroidism, a common cause of hypercalcemia, is confirmed by elevated ionized calcium concentrations and elevated parathyroid hormones. Intact PTH levels are also used to assess and manage other metabolic bone disorders, including osteoporosis and renal osteodystrophy. The measurement

of PTH using two-site immunoassays provides a more accurate assessment of parathyroid tissue secratory status, especially in patients with renal impairment.

III. Specimen Collection and Handling

EDTA plasma (lavender top) or Serum (red or gold top) is the sample of choice. Centrifuge red or gold top tubes within 1hr after collection and lavender top tubes immediately after collection. **For Intra-operative PTH always collect lavender top tubes and perform testing immediately after centifugation.**

The type of specimen used (serum or EDTA plasma) may influence Intact PTH measurements. During routine monitoring of iPTH levels, to avoid bias in results, use the same specimen type throughout the monitoring period.

Patient Sample Stability is shown in the table:

Temperature	EDTA Plasma	Serum
Room temp	8 hrs	4 hrs
4°C	72 hrs	48 hrs
-20°C	2 months	2 months
-70°C	8 months	Not Tested

For Reference Lab and other outside clients aliquots of refrigerated or frozen samples must meet the patient sample stability table shown above, otherwise the sample will be rejected and the test request canceled.

In case the Centaur is out of service , the Immulite 1000 rapid PTH is the back up method to use for intraoperative PTH samples. If any of the intraoperative samples were performed on the Centaur before taken out of service, repeat those samples on the back up method, Immulite. All of the patient's intraoperative samples must be performed on the same analyzer.
When used intraoperatively to verify a decline in PTH production after resection, it is recommended that two or more samples be collected at least 5–10 minutes apart.

The sample volume pipetted for the PTH-INT assay is 200 ul, excluding the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. Ensure samples are free of fibrin, particulate matter, and/or bubbles prior to placing on system.

IV. Reagents

1. ADVIA Centaur Intact PTH primary reagent pack
   a. Lite Reagent – *Stable until expiration date at 2-8°C. Onboard, refer to Onboard Stability and Calibration Interval.*
   5 ml acridinium ester-labeled polyclonal goat antihuman PTH (1-34 N-

terminal) antibody (~1 µg/mL) in phosphate buffered saline with goat IgG, bovine gamma globulin, bovine serum albumin, and preservatives.

b. Solid Phase- *Stable until expiration date at 2-8°C. Onboard, refer to Onboard Stability and Calibration Interval.*
20.0 ml biotinylated polyclonal goat anti-human PTH (39-84 region) antibody (~3 µg/mL) and streptavidin (~0.4 mg/mL) covalently coupled to paramagnetic latex particles in phosphate buffered saline with goat IgG, bovine gamma globulin, bovine serum albumin, and preservatives.

c. Multi-Diluent 11- *Stable until expiration date at 2-8°C or 28 consecutive days after accessing the ancillary reagent pack.*
5.0 mL tris buffer with goat serum with protein stabilizers and preservatives. Store at 2-8°C until the expiration date on the label.

A. Precautions

1. This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.
2. Prewarming or bringing the reagents to room temperature before use is not required.
3. Septum caps are required on all reagents loaded on reagent carousel.
4. Do not use kit components beyond expiration date.
5. Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics.
6.

B. Loading Reagents

1. Ensure that the system has sufficient primary and ancillary reagent packs.
2. **CAUTION:** Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and re-suspended.
3. Load the Ready Pack reagent packs in the primary reagent area using the arrows as a placement guide. The system automatically mixes the primary reagent packs to maintain homogenous suspension of the reagents.

C. Onboard stability

1. The ADVIA Centaur intact PTH assay has an onboard stability of 28 days.
2. Discard the primary reagent packs at the end of the onboard stability interval
3. Do not use reagents beyond the expiration date.

V. Instrumentation/Equipment

The ADVIA CENTAUR system is an automated, immunoassay analyzer that offers optimal productivity and efficiency. No-pause reloading of reagents, samples, and supplies means that the system is always ready to process samples. All assays use direct chemiluminescent technology. Chemiluminescence is a chemical reaction that emits energy in the form of light. When used in combination with immunoassay technology, the light produced by the reaction indicates the amount of analyte in the sample. Direct chemiluminescent reactions directly measure the light energy without

the use of added steps or amplifying molecules. The ADVIA Centaur assays use acridinium ester as the chemiluminescent label, since it does not require the addition of a catalyst or substrate.

When the sample start key is pressed, the barcode labels on the sample cups are read, sample is aspirated, reagent is dispensed, and the assay process begins. Particles are magnetically separated in the cuvette incubation ring. The addition of hydroxyl groups to complete the flash reaction is accomplished by the addition of Reagent 1 & 2; Acid and Base. The chemiluminescent reaction occurs in the luminometer. The photomultiplier tube measures the chemical light reaction that takes place.

There is one (1) main system operation key on the ACS:CENTAUR, the "**Sample Start button**". Pressing this key performs the following actions:

1. Homes the subsystems.
2. The system starts specimen sampling.
3. If the start button is pressed while the instrument is running, it stops sampling additional specimens, however it continues to process the specimens in the incubation ring.

Additional Equipment and Supplies

Reagent Water
Sample cups / tubes
Cuvettes
Sample tips
Reagent 1 (0.5% H2O2; 0.1N HNO3)
Reagent 2 (less than 0.25N. Sodium Hydroxide and surfactant)
ACS:CENTAUR Cleaning Solution
ACS:CENTAUR primary and ancillary reagents.

VI. Calibration

The ADVIA Centaur system uses a Master Curve and a two-point operator initiated calibration to calibrate assays. The Master Curve is established as part of the manufacturing process for each assay lot number.

The ADVIA Centaur intact PTH assay requires a Master Curve calibration when using a new lot number of Lite Reagent and Solid Phase. For each new lot number, use the barcode reader to enter the Master Curve values on the system. The Master Curve Card contains the Master Curve values. Refer to the system operating instructions for more information.

A two-point calibration must be performed at regular, assay specific intervals. Replicates for two calibrators of known value are processed. If the calibrators meet defined validity criteria, the system is adjusted. Refer to the Centaur Operating Procedures for calibration procedure.

A. Calibration Frequency

Two-point calibration of the intact PTH assay is required:

1. every 14 days
2. when changing lot numbers of primary reagent packs
3. when replacing system components
4. when QC results are unacceptable

B. Calibration Material

The intact PTH assay is calibrated with iPTH Calibrators, Low and High.

1. Reconstitute each vial with 1.0 ml reagent grade water using a volumetric pipet.
2. Let the calibrator stand for 30 minutes at room temperature.
3. Gently swirl and invert the vials until homogenous.
4. Each lot of calibrators contains a Calibrator Assigned Value card. Calibrator values may be entered by using the barcode wand or the keyboard.
5. Affix the Low and High Calibrator barcode labels to the appropriate calibrator sample cups so that the system recognizes the sample as a calibrator.
6. Refer to the Centaur Operating Procedures for scheduling and running a calibration.

C. Stability / Storage

1. Lyophilized calibrator is stored at **-20°C** until expiration date on the vial
2. Reconstituted calibrator is stable at room temp. or onboard for **4 hrs**.

D. Precautions

1. Sodium Azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides.
2. Potential biohazard, human and/or other biological source material. Handle as if potentially infectious, according to established good laboratory practices.
3. Do not return any calibrators back into the vials after calibration because evaporation can occur, which may affect performance.
4. Dispose of any calibrator remaining in the sample cups after 4 hours.
5. Do not refill calibrator sample cups when the contents are depleted. If required, dispense fresh calibrators into new cups.

VII. Quality Control

A. QC product: BioRad Liquichek Specialty Immunoassay Control, Levels 1, 2, & 3. BioRad ECS Division, Anaheim, CA/

B. Preparation/handling

1. Stored frozen. This QC product is stable until the expiration date on the label when stored unopened at –20 to -70°C.
2. Allow the frozen control to stand at room temperature until it is completely thawed. Before sampling, gently swirl the vial several times to ensure homogeneity. Promptly replace the stopper and refrigerate at 2 to 8°C
3. Once thawed and opened, all analytes are stable for thirty days when stored tightly capped at 2 to 8°C, except for PTH-INT which is stable for 23 days.
4. Do not use past the expiration date.

5. Discard the vial if there is evidence of microbial contamination or excessive turbidity in the product.

C. Frequency

All three levels of quality control are run on each day on first shift.

D. Acceptability Criteria

Acceptability of QC is determined by the lab internal QC policy. Corrective action for out-of control QC is outlined in the QC policy and actions taken must be documented in the LIS system. No patient results may be released until QC results are acceptable.

VIII. Procedure

1. Performed daily. Frozen patient aliquots will be thawed by Centaur operator.
2. Prepare the sample container for each sample, ensuring that a barcode label is affixed.
3. Samples can be loaded on the Aptio in the appropriate lane or if front loaded, use the appropriately coded sample racks for the type of sample tube to be used:
   a. Position 1 – aliquot tube (blue screw cap)
   b. Position 2 – primary sample tube
   c. Position 3 – sample cup (Siemens)
4. Load each sample tube into a rack, ensuring that the barcode is visible through the slot in the rack.
5. Place the rack(s) in the entry queue.
6. Press 'START' **only** if the system is not currently 'In Process'. The analyzer will read the barcode label and run the appropriate tests.
7. For the PTH assay, the system automatically performs the following steps:
   a. dispenses 200 ul of sample into a cuvette.
   b. dispenses 50 ul of Lite Reagent and incubates for 5.0 min. at 37°C.
   c. dispenses 200 ul of Solid Phase and incubates 2.5 min. at 37°C.
   d. separates, aspirates, and washes the cuvettes with reagent water.
   e. dispenses 300 ul each of Acid Reagent and Base Reagent to initiate the chemiluminescent reaction.
   f. reports results.

IX. Reporting Results

A. Calculations

The system automatically reports serum PTH-INT results in pg/ml. For detailed information about how the system calculates results, refer to the system operating instructions.

B. Limitations of Procedure

1. Assay range (AMR): 6.3 – 1900 pg/ml
   (manufacturer's stated 2.5-1900.0 pg/mL)
   Results less than 6.3 pg/ml, report as "< 6.3 pg/ml"

2. Automatic dilution: The system is programmed to perform an automatic repeat with a x5 dilution for patient samples reading >1900 pg/ml. If a repeat test is required, the system automatically schedules and performs the repeat test. No operator intervention is required, as the sample racks are held in the in process queue until results are completed.

3. Manual dilution: Not normally needed. A smaller dilution, if needed, may be made by using Multi-Dil 11.

C. Reference Interval

11-80 pg/mL

D. Reporting

1. **Analytical measurement range (AMR):** 6.3 – 1900 pg/ml

2. **Clinical reportable range (CRR):** 6.3 – 9,500 pg/ml (using x5 autodilute)

   Report results less than 6.3 as "< 6.3 pg/ml".

X. Procedural Notes/Problem-Solving Tips

1. It is extremely important to ensure that patient samples have been handled and stored correctly. Incorrect handling of samples will result in a loss of intact PTH.
2. Expedite processing and analysis of intraoperative PTH samples. The calcium component should be entered as 'NA', and results must be phoned ASAP. There will usually be a preoperative specimen as well as a postoperative specimen.
3. Interpretation of intact PTH values should always take into account serum calcium results and the interrelationship between these two elements in various disorders involving PTH and calcium.
4. The ADVIA Centaur Intact PTH assay was standardized using purified human PTH (1-84). Value assignment was based on adjustment to an alternate method protocol. The ADVIA Centaur Intact PTH standards are traceable to WHO Standard Preparation 79/500. The average recovery of WHO material is 73% over the full range of the assay. Assigned values for calibrators are traceable to this standardization.
5. Patient samples with high intact PTH levels can cause a paradoxical decrease in RLUs (high dose hook effect). In this assay, patient samples with PTH levels as high as 100,000 pg/ml do not demonstrate a high dose hook effect.
6. Lipemic (<3000 mg/dL of triglycerides), icteric (<40 mg/dL of bilirubin), and

hemolyzed (<200 mg/dl of hemoglobin) demonstrate ≤10% change in results.

7. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed.

XI. Performance Characteristics

1. Analytical Sensitivity: 2.5pg/mL
2. Dilution Recovery: Five human plasma samples in the range of 1040.2 to 1824.1 pg/mL of iPTH were diluted 1:2, 1:4, and 1:8 with Multi-Diluent 11 and assayed for recovery and parallelism. The recoveries ranged from 91.3 to 116.3% with a mean of 99.8%
3. Spiking Recovery: Varying amounts of iPTH were added to five samples with endogenous iPTH levels of 37.8 to 178.7 pg/mL. The recoveries ranged from 91.0 to 123.6% with a mean of 105.0%
4. Precision: Three samples were assayed 12 times, in each of 12 runs, on 3 systems (n=144 for each sample), over a period of 4 days by the manufacturer. The following results were obtained:

Mean pg/mL	Within Run %CV	Run to Run % CV	Total % CV
40.4	5.2	5.8	7.8
223.8	3.4	1.5	7.0
859.3	3.5	2.8	4.6

XII. References

1. Intact PTH (iPTH) Instructions for Use, Siemens Centaur and Centaur XP, Document ID 10492444_EN, Rev. B, 5/2011. Siemens Healthcare Diagnostics.
2. Intact PTH Calibrator Instructions for Use, Document 10492392 Rev.A, 08/2010. Siemens Healthcare Diagnostics.
3. Intact PTH(iPTH) Instructions for use: Siemens Centaur and Centaur XP, Document 10699545_EN Rev D, 2014-08.

POLICY CREATION :		
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REVISION HISTORY (began tracking 2011)			
Rev	**Description of Change**	**Author**	**Effective Date**
1	Added revision history chart, revised for new LIS system, changes in QC	R. Pavlacic	9/9/11
2	Revised due to new biotin free assay method.	T. King	12/7/2011
3	Title/header formatting changes, added manufacturer sens. and assay range, smaller dilution protocol addition.	R Pavlacic	2/1/12
4	Updated Centaur to Centaur XP, removed downtime notification associated with two centaurs	D Roth/ S Burton	6/03/14
5	Updated quality control prep and handling	D Roth/ S Burton	3/30/16
6	Updated LIS code due to Sunquest install.	M. Greer	6/1/16
7	Updated from XP to XPT, Added sample can be loaded on Aptio lane	A.Gibbs	2/13/17
8	Added back up method and all of the patient's intraoperative samples must be performed on the same analyzer.	A.Gibbs	05/26/17

Reviewed by

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				Theresa R King	1/10/12	Donald J Frederick	1/10/12
				Theresa R King	5/8/12	Donald J Frederick	5/15/12
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