DXI (PTHIO) PARATHYROID HORMONE INTRA-OPERATIVE

☒ St. Joseph Medical Center Tacoma, WA ☐ St. Clare Hospital Lakewood, WA ☐ St. Elizabeth Hospital Enumclaw, WA
☐ St. Francis Hospital Federal Way, WA ☒ St. Anthony Hospital Gig Harbor, WA ☐ Highline Medical Center Burien, WA ☐ PSC

PURPOSE

To provide instruction on how to perform Intra-Operative PTH Intact levels using the Beckman Coulter DXI Immunoassay system.

PRINCIPLE

The PTH reagent, when used in conjunction with the Beckman DXI System and Access Calibrators, is intended for quantitative determination of Intact PTH concentration in human serum or plasma.

BACKGROUND

Clinical Significance

Parathyroid hormone (PTH) is synthesized by the chief cells of the parathyroid glands and stored into dense neuroendocrine-type secretory granules, awaiting secretion. Intact PTH is an 84 amino acid polypeptide with a molecular mass of approximately 9.43 kilodaltons. After secretion PTH undergoes rapid proteolysis to generate various circulating C-terminal fragments. Some of these fragments re-enter the bloodstream and are cleared primarily by glomerular filtration, an important route for PTH clearance. The intact and biologically active peptide has a half-life in the circulation of less than 5 minutes.

PTH plays a crucial role in maintaining calcium homeostasis and its measurement is an important aid in the diagnosis of calcium related disorders. In healthy individuals, PTH secretion responds to small alterations in plasma ionized calcium concentration within seconds. Abnormally low ionized calcium concentrations trigger PTH secretion, whereas rising levels of extracellular calcium reduce PTH secretion through a negative feedback mechanism.

PTH regulates calcium levels by concerted effect on three principal organs: bone, intestinal mucosa and kidney. The effect of PTH on intestinal calcium is indirect, resulting from renal production of the intestinally active vitamin D metabolite, 1,25-dihydroxyvitamin D. In the kidney, PTH stimulates calcium reabsorption and inhibition of phosphate reabsorption from the renal tubules. Eventually PTH promotes osteoclastic bone resorption and release of calcium and phosphate from bone.

In patients with disorders of calcium metabolism, quantitative determination of circulating PTH may assist in the differential diagnosis of hypercalcemia and hypocalcemia. In hypercalcemia due to primary hyperparathyroidism or ectopic PTH secretion (pseudo hyperparathyroidism), most patients have increased PTH levels. By contrast in hypercalcemia due to malignancy or other causes, the concentration of PTH in the circulation is typically low, either below or towards the low end of the reference range for apparently healthy individuals.

Secondary hyperparathyroidism is a compensatory hyperfunctioning of the parathyroid glands caused by hypocalcemia or peripheral resistance to PTH. It is typically caused by renal failure and leads to elevated PTH levels. Chronic overproduction of PTH in renal failure contributes to the spectrum of bone disease, which is also termed renal osteodystrophy. The National Kidney Foundation (NKF) has published clinical practice guidelines addressing bone metabolism for the management of chronic kidney disease. It recommends that

serum levels of calcium, phosphorus and PTH be measured periodically in all patients with chronic kidney disease. Since this condition is a complex and multifactorial disease, PTH results should be interpreted in light of all the information available to the clinician.

Hypoparathyroidism is an uncommon congenital or acquired condition in which PTH secretion is deficient or absent. In most cases, hypoparathyroidism follows parathyroidectomy or thyroidectomy. Pseudohypoparathyroidism is a rare disorder and describes hereditary conditions that cause end organ resistance to PTH.

Rapid intraoperative PTH measurement in patients undergoing parathyroidectomy has also been described. Based on a review of the literature, the National Academy of Clinical Biochemistry is in the process of publishing laboratory medicine practice guidelines for the use of intraoperative PTH measurements. These guidelines recommend use of intraoperative PTH testing for patients undergoing surgery for primary hyperparathyroidism and strongly recommend use in minimally invasive or directed procedures.

Methodology

The Access Intact PTH assay is a two-site immunoenzymatic (“sandwich”) assay. A sample is added to a reaction vessel, along with a monoclonal anti-PTH antibody conjugated to alkaline phosphatase, TRIS buffered saline with proteins and paramagnetic particles coated with a goat polyclonal anti-PTH antibody. After incubation in a reaction vessel, materials bound to the solid phase are held in a magnetic field while unbound materials are washed away. Then, the chemiluminescent substrate Lumi-Phos* 530 is added to the vessel and light generated by the reaction is measured with a luminometer. The light production is directly proportional to the concentration of PTH in the sample. The amount of analyte in the sample is determined from a stored, multi-point calibration curve.

RELATED DOCUMENTS

R-PO-CH-0810	Quality Control Program General Laboratory
R-PO-CH-0809	Quality Control Westgard Rules Statistics
R-PR-AD-0540	Specimen Rejection/Cancellation Protocol
J-F-CG-0824	DXI & Access Controls
J-F-CH-0825	DXI Calibrators
M-F-CH-0820	Chemistry Controls
M-F-CH-0826	Chemistry Calibrators
R-F-CH-2000	DXI & Access (AMR) Analytical Measurement Range

SPECIMEN

Type of Specimen

Biological fluid samples should be collected in the same manner routinely used for any laboratory test. Freshly drawn serum or plasma (EDTA and heparin) is the preferred specimen.

Specimen Storage and Stability

1. Tubes of blood are to be kept closed and in a vertical position at all times. It is recommended that the serum or plasma be physically separated from contact with cells within two hours of the time of collection.

2. Separated serum or plasma should not remain at room temperature longer than 8 hours. If assays are not completed within 8 hours, serum or plasma should be stored at +2°C to +8°C. If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples should be frozen at -15°C to -20°C. Frozen samples should be thawed only once. Analyte deterioration may occur in samples that are repeatedly frozen and thawed.

3. Avoid assaying lipemic or hemolyzed samples.

Sample Type	Volume	Sample Stability
Plasma	0.5mL	• Room Temp 8 hours • Refrigerated 48 hours • Frozen 3 months.
Serum		• Separate serum from cells within 2 hours. • Room Temp 4 hours • Refrigerated 8 hours • Frozen 3 months

Criteria for Unacceptable Specimens

Refer to the PROCEDURAL NOTES section of this chemistry information sheet for information on unacceptable specimens. See also Related Documents: Specimen Rejection/Cancellation Protocol

Sample Volume

The optimum volume, when using a 0.5 mL sample cup, is 0.3 mL of sample. For optimum primary sample tube volumes and minimum volumes, refer to the Primary Tube Sample Template for your system.

- The Operating room staff will draw one full lavender top (EDTA) or green top (heparin) tube for the first baseline sample. Sample should be labeled with patient identification, and exact collection time/date of draw. Typically, a baseline specimen (baseline #1) will be drawn at the start of surgery
- Samples should be drawn at 5 and 10 minutes post-resection of the hyperfunctioning parathyroid tissue. Additional samples may be necessary.
- At least 50% reduction in PTH value should be observed when the highest baseline sample is compared to the post-resection samples.

REAGENTS

Access Intact PTH Reagent Pack
Cat. No. A16972: 100 determinations, 2 packs, 50 tests/pack.

Reactive Ingredients	
Paramagnetic particles coated with goat anti-PTH antibody suspended in TRIS buffered saline with bovine serum albumin (BSA), surfactant, < 0.1% sodium azide, 0.1% ProClin** 300.	R1a
TRIS buffered saline with block ACE, protein (mouse, goat), surfactant, < 0.1% sodium azide, 0.1% ProClin** 300.	R1b
Mouse monoclonal anti-PTH alkaline phosphatase conjugate in ACES buffered saline with BSA, surfactant, < 0.1% sodium azide, 0.1% ProClin** 300.	R1c

Reagent Preparation

Used for both the Routine and Intraoperative modes. Provided ready to use.
Reagent Storage and Stability

Store upright and refrigerate at 2 to 10°C. Refrigerate at 2 to 10°C for a minimum of two hours before use on the instrument. Stable until the expiration date stated on the label when stored at 2 to 10°C. Stable at 2 to 10°C for 28 days after initial use. Signs of possible deterioration are a broken elastomeric layer on the pack or control values out of range.

If the reagent pack is damaged (i.e., broken elastomer), discard the pack. All antisera are polyclonal unless otherwise indicated.

CALIBRATION

Calibrator Required

Access Intact PTH Calibrators
Cat. No. A16953: PTH Reconstitution Buffer (RB), 2 vials, 4.0 mL/vial; S0–S5, 1.0 mL/vial

RB: Buffered protein (bovine) matrix, 0.5% Pro Clin** 300.

S0: PBS buffer, bovine serum albumin (BSA), surfactant, < 0.1% sodium azide.

S1–S5: Approximately 10, 60, 300, 1500 and 3500 pg/mL (1.1, 6.4, 31.8, 159.0 and 371.0 pmol/L) PTH (synthetic antigen), respectively in PBS buffer with BSA, surfactant, < 0.1% sodium azide.

Calibration Card: 2
For the Access Intact PTH assay, one calibration card is provided for Routine Mode and a separate calibration card is provided for the Intraoperative Mode.

Calibrator Preparation

Used for both the Routine and Intraoperative modes. Calibrators and Reconstitution Buffer are **single use only**. S0–S5 calibrators are provided lyophilized. Reconstitute each calibrator vial volumetrically with 1.0 mL **PTH Reconstitution Buffer**. Allow 30 minutes for dissolution. Mix gently before use.

Calibrator Storage and Stability

Use reconstituted calibrators within 2 hours if stored at 18–25°C. Use reconstituted calibrators within 10 hours if stored at 2–10°C. Lyophilized calibrators and PTH Reconstitution Buffer are stable until the expiration date stated on the label when stored at 2 to 10°C. Signs of possible deterioration are control values out of range or failure of calibrators to completely reconstitute. Refer to calibration card for exact concentrations.

Calibration Information

Quantitative assay calibration is the process by which samples with known analyte concentrations (i.e., assay calibrators) are tested like patient samples to measure the response. The mathematical relationship between the measured responses and the known analyte concentrations establishes the calibration curve. This mathematical relationship, or calibration curve, is used to convert RLU (Relative Light Unit) measurements of patient samples to specific quantitative analyte concentrations.

An active calibration curve is required for all tests. For the Access Intact PTH assay, calibration is required every 28 days. Refer to the appropriate system manuals and/or Help system for information on calibration theory, configuring calibrators, calibrator test request entry, and reviewing calibration data.

The Access Intact PTH Calibrators are provided at six levels – zero and approximately 10, 60, 300, 1500 and 3500 pg/mL (1.1, 6.4, 31.8, 159.0 and 371.0 pmol/L). Assay calibration data are valid up to 28 days.

Calibrators run in duplicate.

For the Access Intact PTH assay, one calibration card is provided for Routine Mode and a separate calibration card is provided for the Intraoperative Mode.

Other Required Materials

Access Substrate: 4 x 130 mL

Provided ready to use. Refer to the following chart for storage conditions and stability. An increase in substrate background measurements may indicate instability.

SUBSTRATE		
Condition	**Storage**	**Stability**
Unopened	2 to 8°C	Until expiration date stated on the label
Equilibration prior to use (unopened)	15 to 30°C (room temperature)	Minimum 18 hours Maximum 14 days
In use (opened)	Internal substrate supply position	Maximum 5 days
In use (opened)	External fluids tray substrate position	Maximum 14 days

R2 Substrate: Lumi-Phos 530 (buffered solution containing dioxetane Lumigen* PPD, fluorescer, and surfactant).

Unicel DxI Wash Buffer II

Provided ready to use. Stable until the expiration date stated on the label when stored at room temperature (15 to 30°C). An increase in substrate background measurements or increased relative light units for the zero calibrators in “sandwich”-type assays may indicate instability.

R3 Wash Buffer: TRIS buffered saline, surfactant, < 0.1 sodium azide, and 0.1% ProClin 300.

Access Sample Diluent A: 4 mL/vial

The analyte level in patient samples may exceed the level of the specific S5 calibrator. If a quantitative value is required, it will be necessary to dilute the samples in order to determine the analyte concentration.

Provided ready to use. Allow the contents to stand for 10 minutes at room temperature. Mix gently by inverting before use. Avoid bubble formation. Stable until the expiration date stated on the vial label when stored at 2 to 10°C.

Samples can be accurately measured within the analytic range of the lower limit of detection and the highest calibrator value of the specific assay. If a sample contains more analyte than the stated value of the S5 calibrator, dilute the sample following dilution instructions in the specific assay labeling under “Limitations of the Procedure" in the reagent pack section. Refer to the appropriate system manuals and/or Help system for instructions on how to enter a sample dilution in a test request.

Access Sample Diluent A: Buffered BSA matrix with surfactant, < 0.1% sodium azide, 0.5% ProClin 300.

Warnings and Precautions

- For *in vitro* diagnostic use.
- Patient samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment, or prior certification. Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.
- Substrate is sensitive to air exposure. Keep tightly closed at all times. Do not pool bottles of substrate.
- Xi. Irritant: 0.1% ProClin 300.
  R 43: May cause sensitization by skin contact.
  S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.
- The Material Safety Data Sheet (MSDS) is available upon request.

QUALITY CONTROL

See Related Documents M-F-CH-0820 Chemistry Controls & J-F-CG-0824 DXI & Access Controls

STEPS

1. If necessary, load the reagent onto the system. Use PTHIO as the test name for the Access Intact PTH assay Intraoperative Mode. The same reagent pack is used for both modes.

2. After reagent load is completed, calibration may be required.

3. Program controls for analysis.

4. After loading controls onto the system, follow the protocols for system operation. Refer to the appropriate system manuals and/or Help system for preparation and operation.

RESULTS

Patient test results are determined automatically by the system software using a smoothing spline math model. The amount of analyte in the sample is determined from the measured light production by means of the stored calibration data. Patient test results can be reviewed using the appropriate screen. Refer to the appropriate system manuals and/or Help system for complete instructions on reviewing sample results.

PERFORMANCE CHARACTERISTICS

Reference Range

No Reference range established. The intra-operative parathyroid hormone results will be used as a monitoring tool during surgery only. It is not intended for the diagnosis or management of malignancy.

Analytic Range

Sample Type	Conventional Units
Serum or Plasma	6 – 3500 pg/mL

Samples with concentrations exceeding the high end of the analytical range should be diluted with Sample A Diluent and reanalyzed. The appropriate dilution factor should be applied to the reported result.

Reporting results outside of the analytical range

Lower limit of range: serum / plasma	6 pg/mL	Result below 6, report as <6 pg/mL
Upper limit of range: serum / plasma	3500 pg/mL	Results >3500 pg/mL should be diluted with Sample A Diluent, reanalyzed and dilution factor applied. The maximum allowable dilution is X2. Results >7,000 are reported as >7,000 pg/mL

LIMITATIONS

1. For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the patient sample. Patients who have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in patient samples. Such interfering antibodies may cause erroneous results. Carefully evaluate the results of patients suspected of having these antibodies.

1. The Access Intact PTH results should be interpreted in light of the total clinical presentation of the patient, including: symptoms, clinical history, data from additional tests and other appropriate information.

2. The Access Intact PTH assay does not demonstrate any “hook” effect up to 250,000 pg/mL (26,500 pmol/L).

3. The Intraoperative Mode of the Access Intact PTH assay is not recommended for use in routine PTH testing. Performance characteristics for cross reactivity with metabolized forms of PTH have not been established.

4. For Intraoperative Mode testing with the Access Intact PTH assay in patients undergoing parathyroidectomy for primary hyperparathyroidism, the following practices are recommended:

    - Baseline samples should be drawn at pre-operation/exploration and pre-excision.
    - Samples should be drawn at 5 and 10 minutes post-resection of the hyperfunctioning parathyroid tissue. Additional samples may be necessary.
    - At least a 50% reduction in PTH value should be observed when the highest baseline sample is compared to the post-resection samples.

LIMITATIONS: INTRAOPERATIVE MODE

1. The following drugs/interferents were added to an EDTA plasma sample pool containing approximately 60 pg/mL PTH. Each drug/interferent was tested at a minimum of the concentration listed below. All PTH values obtained in the presence of each drug/interferent were within ± 10% of the control values, indicating that these substances do not interfere with the assay

Drug/interferent	Concentration tested	% Interference
Bilirubin, conjugated	20 mg/dL INDEX of 14	1.1
Bilirubin, unconjugated	20 mg/dL INDEX of 14	2.2
Triolein (Lipemia)	3000 mg/dL INDEX of 10	6.1
Cholesterol	500 mg/dL	5.3
Hemoglobin	500 mg/dL INDEX of 10	-0.3
Human serum albumin	49 g/L	-9.9
Acetaminophen	20 mg/dL	-0.3
D-biotin	100 µg/L	-1.3
Heparin	8000 IU/dL	-2.0
Ibuprofen	40 mg/dL	0.2
Pamidronate	10 µg/mL	-0.2
Propofol	2 µg/mL	-4.0
Salicylic acid	50 mg/dL	2.0

2. The lowest detectable level of PTH distinguishable from zero (Access Intact PTH Calibrator S0) with 95% confidence is 6 pg/mL.

3. The term functional sensitivity was originally used to define the lowest point in a TSH assay measuring range where results could be derived with a consistently attainable total imprecision of 20% CV.(12) The functional sensitivity, as determined by total imprecision of 20% CV, was found to be < 8 pg/mL.

PROCEDURAL NOTES

1. Refer to the appropriate system manuals and/or Help system for a specific description of installation, start-up, principles of operation, system performance characteristics, operating instructions, calibration procedures, operational limitations and precautions, hazards, maintenance, and troubleshooting.

2. Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the instrument. Do not invert open (punctured) packs.

3. Use fifty-five (55) µL of sample for each determination in addition to the sample container and system dead volumes. Refer to the appropriate system manuals and/or Help system for the minimum sample volume required.

4. The system default unit of measure for sample results is pg/mL. To change sample reporting units to the International System of Units (SI units), pmol/L, refer to the appropriate system manuals and/or Help system. To manually convert concentrations to the International System, multiply pg/mL by multiplication factor 0.106.

REFERENCES

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DOCUMENT APPROVAL **Purpose of Document / Reason for Change:**			
Updated to new format, added max dilution and index information. minor updates to match Beckman procedure.			
Committee Approval Date	☒ Date: 1/8/2015 ☐ NA – revision of department-specific document which is used at only one facility	**Medical Director Approval** *(Electronic Signature)*	Katie Wilkinson, MD 8/25/15