Hormone

ichroma™ PTH

INTENDED USE

ichroma™ PTH is a fluorescence immunoassay (FIA) for the quantitative determination of PTH (parathyroid hormone) in human whole blood/serum/plasma. It is useful as an aid in the differential diagnosis of hyperparathyroidism or hypoparathyroidism.

For *in vitro* diagnostic use only.

INTRODUCTION

Parathyroid hormone is a single-chain polypeptide hormone consisting of 84 amino acids with an approximate molecular weight of 9.5 kDa and is secreted by the chief cells of the parathyroid gland. It is a major regulator of bone remodeling and participates in the homeostatic response to alterations in plasma calcium concentrations. [1]

PTH is biosynthesized as a pre-proparathyroid hormone, a large molecular precursor consisting of 115 amino acids. After two intra-cellular proteolytic cleavage steps, the parathyroid gland secretes the final active form consisting of an 84 amino acid peptide, called intact form. Intact PTH is biologically active and clears very rapidly from the circulation with a half-life of less than four minutes.

In healthy individuals, regulation of parathyroid hormone secretion normally occurs via a negative feedback action of serum calcium on the parathyroid glands. Intact PTH assays are important for the differentiation of primary hyperparathyroidism from other (non-parathyroid-mediated) forms of hypercalcemia, such as malignancy, sarcoidosis, and thyrotoxicosis. The measurement of parathyroid hormone is the most specific way of making the diagnosis of primary hyperparathyroidism. In the presence of hypercalcemia, an elevated level of parathyroid hormone virtually establishes the diagnosis. In over 90% of patents with primary hyperparathyroidism, the parathyroid hormone will be elevated. The most common other cause of hypercalcemia, namely hypercalcemia of malignancy, is associated with suppressed levels parathyroid hormone or PTH levels within the normal range. [2,3,4]

PRINCIPLE

The test uses a sandwich immunodetection method.

The detector antibodies in buffer bind to antigens in the sample, forming antigen-antibody complexes, and migrate onto nitrocellulose matrix to be captured by the other immobilized streptavidin on a test strip.

More antigens in the sample will form more antigen-antibody complexes which lead to stronger fluorescence signal by detector antibodies, which is processed by the instrument for ichroma™ tests to show PTH concentration in the sample.

COMPONENTS

ichroma™ PTH consists of 'cartridges', 'detector tubes', and 'detector diluent'.

- The cartridge contains the membrane called a test strip which has streptavidin at the two test lines, and chicken IgY at the control line. All cartridges are individually sealed in an aluminum foil pouch containing a desiccant, and they are further packaged in a box.
- The detector tube has a granule containing anti-PTH-fluorescence conjugate, anti-PTH-biotin conjugate, anti-chicken IgY-fluorescence conjugate and sodium azide as a preservative in phosphate buffered saline (PBS). All detector tubes are packed in a pouch.
- The detector diluent contains tergitol and tween 20 as detergent and sodium azide as a preservative in HEPES Buffer, and it is pre-dispensed in a vial. The detector diluent is packed in a box.

WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use only.
- Follow instructions and procedures described in this 'Instructions for use'.
- Use only fresh samples and avoid direct sunlight.
- Lot numbers of all the test components (cartridge, detector tube, detector diluent, and ID chip) must match each other.
- Do not interchange the test components between different lots or use the test components after the expiration date, either of which might yield incorrect test result(s).
- Do not reuse cartridge or detector tubes. A cartridge should be used for testing one sample only. A detector tube should be used for processing of one sample only.
- The cartridge should remain sealed in its original pouch until just before use. Do not use a cartridge, if the pouch is damaged or has already been opened.
- Frozen sample should be thawed only once. For shipping, samples must be packed in accordance with local regulations. Sample with severe hemolysis and/or hyperlipidemia must not be used.
- If test components and/or sample are stored in refrigerator, then allow cartridge, detector tube, detector diluent and sample to be at room temperature for approximately 30 minutes before use.
- The instrument for ichroma™ tests may generate slight vibration during use.
- Used cartridges, detector tubes, detector diluent and pipette tips should be handled carefully and discarded by an appropriate method in accordance with relevant local regulations.
- The detector tube and the detector diluent contain sodium azide (NaN_3), and it may cause certain health issues like convulsions, low blood pressure, low heart rate, loss of consciousness, lung injury and respiratory failure. Avoid contact with skin, eyes, and clothing. In case of contact, rinse immediately with running water.
- No Biotin interference was observed in **ichroma™ PTH** when biotin concentration in the sample was below 100ng/mL. If a patient has been taking biotin at dosage of more than 0.03 mg a day, it is recommended to test again 24 hours after discontinuation of biotin intake.

- **ichroma™ PTH** will provide accurate and reliable results subject to the below conditions.
  - **ichroma™ PTH** should be used only in conjunction with the instrument for ichroma™ tests.
  - Have to use recommended anticoagulant.

Recommended anticoagulant
K_2 EDTA, Sodium heparin, Lithium heparin

LIMITATION OF THE TEST SYSTEM

- The test may yield false positive result(s) due to the cross-reactions and/or non-specific adhesion of certain sample components to the capture/detector antibodies.
- The test may yield false negative result(s) due to the non-responsiveness of the antigens to the antibodies which is the most common if the epitope is masked by some unknown components, so therefore not being able to be detected or captured by the antibodies. The instability or degradation of the antigens with time and/or temperature may also cause false negative result as it makes the antigens unrecognizable by the antibodies.
- Other factors may interfere with the test and cause erroneous results, such as technical/procedural errors, degradation of the test components/reagents or presence of interfering substances in the test samples.
- Any clinical diagnosis based on the test result must be supported by a comprehensive judgment of the concerned physician in conjunction with clinical symptoms and other relevant test results.

STORAGE AND STABILITY

Storage condition			
Component	Storage Temperature	Shelf life	Note
Cartridge	2 - 30 °C	20 months	Disposable
Detector tube	2 - 30 °C	20 months	Disposable
Detector diluent	2 - 30 °C	20 months	Unopened
	2 - 8 °C	12 months	Opened

- After the cartridge pouch is opened, the test should be performed immediately.

MATERIALS SUPPLIED

REF CFPC-184

Components of **ichroma™ PTH**

- Cartridge box:
  - Cartridge 25
  - Detector tube 25
  - Detector diluent 1
  - ID chip 1
  - Instructions for use 1

MATERIALS REQUIRED BUT SUPPLIED ON DEMAND

Following items can be purchased separately with **ichroma™ PTH.** Please contact our sales division for more information.

- Instrument for ichroma™ tests
  - **ichroma™ II** REF FPRR021
  - **ichroma™ III** REF FPRR037
  - **ichroma™ M2** REF FPRR031
  - **ichroma™ 50 Plus** REF FPRR036
- **Boditech PTH Control** REF CFPO-404

SAMPLE COLLECTION AND PROCESSING

The sample type for **ichroma™ PTH** is human whole blood/serum/plasma.

- It is recommended to test the sample within 6 hours after collection when collected sample is stored at room temperature.
- The samples (serum, plasma) should be separated from the clot by centrifugation within 3 hours after the collection of whole blood.
- The samples (whole blood, serum, plasma) may be stored for a day at 2-8 °C prior to being tested. If testing will be delayed more than a day, samples (serum, plasma) should be frozen at -20 °C.
- The samples (serum, plasma) stored frozen at -20 °C for 3 months showed no performance difference.
- However, the whole blood sample should not be kept in a freezer in any case.
- As a repeated freeze-thaw cycle may affect the test result, do not refreeze previously frozen samples.

TEST SETUP

- Check the contents of **ichroma™ PTH**: Sealed cartridges, detector tubes, a detector diluent, an ID chip and an instructions for use.
- Ensure that the lot number of the cartridge matches that of the detector tube, the detector diluent as well as an ID chip.
- If the sealed cartridges, the detector tube and the detector diluent have been stored in a refrigerator, place them on a clean and flat surface at room temperature for at least 30 minutes before testing.
- Turn on the instrument for ichroma™ tests.
- Insert the ID chip into the 'ID chip port'.

※ **Please refer to the instrument for ichroma™ tests operation manual for complete information and operating instructions.**

TEST PROCEDURE

▶ **ichroma™ II, ichroma™ M2**

Multi test mode / Read now mode

1) Select the sample type (whole blood or serum/plasma) on the screen.
2) Take 120 µL of detector diluent using a pipette and dispense it to the detector tube containing a granule. When the granule form is completely dissolved in the tube, it becomes detection buffer.
   (The detection buffer must be used immediately. Do not exceed 30 seconds.)
3) Take 50 µL of sample (whole blood/serum/plasma/control) using a pipette and dispense it to the detector tube.
4) Close the lid of the detector tube and mix the sample thoroughly by shaking it about 10 times.
   (The sample mixture must be used immediately. Do not exceed 30 seconds.)
5) Take 75 µL of the sample mixture and dispense it into

the sample well of the cartridge.
6) Leave the cartridge at room temperature for 12 minutes.
⚠ *Scan the sample-loaded cartridge immediately when the incubation time is over. If not, it will cause inaccurate test result.*
7) To scan the sample-loaded cartridge, insert it into the cartridge holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
8) Tap the 'Start' button on the instrument for ichroma™ tests to start the scanning process.
(ichroma™ M2 will start the test automatically after inserting.)
9) The instrument for ichroma™ tests will start scanning the sample-loaded cartridge immediately.
10) Read the test result on the display screen of the instrument for ichroma™ tests.

Single test mode / Walk away mode

1) The test procedure is same with the 'Multi test mode 1) – 5)'.
2) Insert the sample-loaded cartridge into the holder of the instrument for ichroma™ tests. Ensure proper orientation of the cartridge before pushing it all the way inside the cartridge holder. An arrow is marked on the cartridge especially for this purpose.
3) Tap the 'Start' button on the instrument for ichroma™ tests.
(ichroma™ M2 will start the test automatically after inserting.)
4) The cartridge goes inside the instrument for ichroma™ tests and will automatically start scanning the sample-loaded cartridge after 12 minutes.
5) Read the test result on the display screen of the instrument for ichroma™ tests.

▶ **ichroma™ III**

1) The test procedure is same with the 'Single test mode'.

▶ **ichroma™ 50 Plus**

1) Insert the tip array in the tip station.
2) Insert the detector tube in the reagent station and cover the reagent station to hold the detector tubes in place.
3) Open the lid of the detector diluent and insert the detector diluent in the diluent station.
4) Insert the cartridge magazine with the cartridges into the magazine station.
5) Insert the sample tube into the blood collection tube rack and load the blood collection tube rack into the sampling station (loading part).
6) Tap the button located in the upper side of the No. of test cartridge region to select the ID chip that you want to use.
7) When the selected cartridge slot is activated, set the number of the detector tube by tapping.
8) Set the number of pipette tips by tapping.
9) Select the sample type (whole blood or serum/ plasma) on the screen.
10) Tap the 'Start' button on the left upper of the main screen to start test.

INTERPRETATION OF TEST RESULT

- The instrument for ichroma™ tests calculates the test result automatically and displays PTH concentration of the test sample in terms of pg/mL.
- Each laboratory should investigate the transferability of the expected values to its own patient population, and if necessary, determine its own reference range.
- Working range: 30 - 3,000 pg/mL
- Cut-off: 65 pg/mL

QUALITY CONTROL

- Quality control tests are a part of the good testing practice to confirm the expected results and validity of the assay and should be performed at regular intervals.
- Quality control tests should also be performed whenever there is any question concerning the validity of the test results.
- Control materials are provided on demand with **ichroma™ PTH**. For more information regarding obtaining the control materials, contact Boditech Med Inc.'s Sales Division for assistance.
(Please refer to the instructions for use of control material.)

PERFORMANCE CHARACTERISTICS

- **Analytical sensitivity**
  - Limit of Blank (LoB) 5.29 pg/mL
  - Limit of Detection (LoD) 14.78 pg/mL
  - Limit of Quantitation (LoQ) 29.88 pg/mL
- **Analytical specificity**
  - Cross-reactivity

  Biomolecules listed in the following table were added to the test sample(s) at concentrations much higher than their normal physiological levels in the blood. **ichroma™ PTH** test results did not show any significant cross-reactivity with these biomolecules, except for the PTH (1-34) fragment.

Cross-reactants	Concentration (pg/mL)
PTH (1-34) fragment	12,000
PTH (39-68) fragment	100,000
PTH (39-84) fragment	100,000
PTH (44-68) fragment	100,000
PTH (53-84) fragment	100,000
PTH (7-84) fragment	300
Beta-Cross Laps	10,000
Calcitonin	100,000
Osteocalcin	50,000
PTH RP (1-34)	100,000

*Note: Heterophilic antibodies in patient samples can cause detection interference by cross-reacting with assay detection components including streptavidin. These interactions may lead to false-positive results.

- Interference

Interferents listed in the following table were added to the test sample at the concentration mentioned below. **ichroma™ PTH** test results did not show any significant interference with these materials.

Interferents	Concentration
Hemoglobin	1000 mg/dL
Bilirubin, unconjugated	0.7 mmol/L
Triglycerides	50 g/L
Ascorbic acid	0.3 mmol/L
Glucose	1000 mg/dL
Cholesterol	400 mg/dL

- **Precision**

- Single-site study

Repeatability (within-run precision)
within-laboratory precision (Total precision)
Lot to lot precision

3 Lots of **ichroma™ PTH** were tested for 20 days. Each standard material was tested 2 times per day. For each test, each material was duplicated.

Single-site study						
PTH	Repeatability		within-laboratory precision		Lot to lot precision	
[pg/mL]	AVG [pg/mL]	CV (%)	AVG [pg/mL]	CV (%)	AVG [pg/mL]	CV (%)
50	49.43	7.6	49.28	7.3	49.39	7.5
93	93.54	7.8	93.77	7.9	93.14	7.5
833	817.37	7.3	828.62	7.7	833.43	7.6

- Multi-site study

Reproducibility

1 Lot of **ichroma™ PTH** was tested for 5 days in 3 different sites (1 person per 1 site, 1 instrument per 1 site). Each standard material was tested 1 time per and 5 replicates per day.

Multi-site study		
PTH	Reproducibility	
[pg/mL]	AVG [pg/mL]	CV (%)
50	49.23	7.8
93	91.88	7.9
833	829.02	7.5

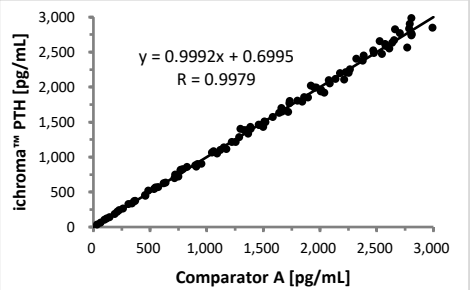
- **Accuracy**

The accuracy was confirmed by testing with 3 different lots of **ichroma™ PTH**. The tests were repeated 10 times at each concentration of the control standard.

PTH [pg/mL]	Lot 1	Lot 2	Lot3	AVG [pg/mL]	Recovery (%)
50.38	51.42	50.88	49.56	50.62	100
209.52	208.29	203.69	216.74	209.57	100
588.57	595.58	593.99	593.52	594.37	101
1157.14	1161.28	1160.67	1115.15	1145.70	99
1750.43	1738.43	1747.01	1766.06	1750.50	100
2341.67	2284.31	2290.19	2279.15	2284.55	98
2862.86	2908.83	2880.42	2862.62	2883.96	101

- **Comparability**

PTH concentrations of 100 clinical samples were quantified independently with **ichroma™ PTH (ichroma™ II)** and **Comparator A** as per prescribed test procedures. Test results were compared, and their comparability was investigated with linear regression and correlation coefficient(R). The regression equation and correlation coefficient are as follows.



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Note: Please refer to the table below to identify various symbols.

	Sufficient for <n> tests
	Read instruction for use
	Use by Date
	Batch code
	Catalog number
	Caution
	Manufacturer
	Authorized representative of the European Community
	In vitro diagnostic medical device
	Temperature limit
	Do not reuse
	This product fulfills the requirements of the Directive 98/79/EC on in vitro diagnostic medical devices

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