

Immunoassay

REF

CME0901 / CME0902 / CME0903 / CME0904 / CME0905

50 tests*1 / 100 tests*1 / 100 tests*2 / 100 tests*5 / 50 tests*2

PTH CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay (CLIA Microparticles) for the quantitative detection of PTH in human serum or plasma (EDTA).

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Key to Graphical Symbols Used

LOT

batch code



use by



manufacturer



contains sufficient for <n> tests

IVD
in vitro diagnostic medical device


temperature limitation

REF

catalogue number



consult instructions for use

EC REP

authorized representative in the European Community

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 OBELIS S.A.
 Bd. Général Wahis, 53
 1030 Brussels
 Belgium

 AUTOBIO DIAGNOSTICS CO., LTD.
 No.87 Jingbei Yi Road
 National Eco & Tech Development Area
 Zhengzhou
 China
 450016


For any technical assistance please contact us in English at:

Email: customerservice@autobio.com.cn

Contact your local dealers for all product related questions in your local language.

Introduction

Parathyroid hormone (PTH), para-thormone or parathyrin, is secreted by the chief cells of the parathyroid glands as a polypeptide containing 84 amino acids. It acts to increase the concentration of calcium in the blood, whereas calcitonin (a hormone produced by the para-follicular cells (C cells) of the thyroid gland) acts to decrease calcium concentration. PTH acts to increase the concentration of calcium in the blood by acting upon the parathyroid hormone 1 receptor (high levels in bone and kidney).¹ PTH half-life is approximately 4 minutes. It has a molecular mass of 9.4 kDa.² Hyperparathyroidism, the presence of excessive amounts of parathyroid hormone in the blood, occurs in two very distinct sets of circumstances. Primary hyperparathyroidism is due to autonomous, abnormal hyper-secretion of PTH from the parathyroid gland, while secondary hyperparathyroidism is an appropriately high PTH level seen as a physiological response to hypocalcaemia.

A low level of PTH in the blood is known as hypo-parathyroidism and is most commonly due to damage to or removal of parathyroid glands during thyroid surgery.

In osteoporotic women, administration of an exogenous parathyroid hormone analogue (teriparatide, by daily injection) superimposed on estrogen therapy produced increases in bone mass and reduced vertebral and non-vertebral fractures by 45 to 65%.³

Measurement Principle

This assay is based upon the one-step sandwich method. The sample, anti-PTH coated microparticles and enzyme labeled anti-PTH are combined. During the incubation, PTH present in the sample is allowed to react simultaneously with the two antibodies, resulting in PTH being sandwiched between the microparticles and enzyme-linked antibodies. After washing, a complex is generated among the microparticles, the PTH within the sample and enzyme-linked antibodies by immunological reactions. Chemiluminescent Substrate is added and the complex catalyzes substrate, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLU. The RLU is proportional to the concentration of PTH in the patient sample.

Materials Provided


1. Calibrators

6 vials each containing 1.0 mL of calibrator A through F. The matrix is Tris-NaCl buffer containing Casein. Contains a selection of preservatives.

Calibrators provided ready to use.

2. Reagent pack

Reagent pack provided ready to use.

	50*1	100*1	100*2	100*5	50*2
Microparticles Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2
Enzyme Conjugate	3.0mL*1	5.5mL*1	5.5mL*2	5.5mL*5	3.0mL*2
Antibody Solution	1.2mL*1	2.3mL*1	2.3mL*2	2.3mL*5	1.2mL*2

• Microparticles Solution

Polyclonal antibodies of PTH coated microparticles in PBS (phosphate buffered saline) buffer containing BSA. Contains a selection of preservatives.

• Enzyme Conjugate

Horseradish peroxidase labeled anti-PTH in a Tris-NaCl buffer containing BSA. Contains a selection of preservatives.

• Antibody Solution

Tris-NaCl buffer containing BSA. Contains a selection of preservatives.

Assay Analyzers on which the kit can be used

- AutoLumo A2000 Plus
- AutoLumo A2000 Plus B
- AutoLumo A1000

The chemiluminescent microparticle immunoassay (CLIA Microparticles) is intended for use on Assay Analyzer which is AutoLumo A2000 Plus, AutoLumo A2000 Plus B or Autolumo A1000.

Materials Required but not Provided

1. Assay Analyzer
2. Reaction vessel(s) for sample and reagent reaction
3. Sample cup(s) or tube(s) for sample containing
4. Diluent Universal
5. Chemiluminescent Substrate
6. System Wash for washing the pipetting needles
7. Wash Buffer used in the washing procedure
8. Distilled water or deionized water

Metrological Traceability of Calibrators

The measurand (analyte) in the PTH Calibrators is traceable to the manufacturer's working calibrators.

Warnings and Precautions

1. For professional use only.
2. Follow the instruction for use carefully. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.
3. Refer to the material safety data sheet and product labeling for any chemical hazards that may be present in this assay.
4. Handle the potentially contaminated materials and wastes safely according to local requirement.
5. CAUTION: This assay contains materials of animal origin. Bovine components originate from countries where BSE has not been reported.
6. Do not smoke, drink, eat or use cosmetics in the working area.
7. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
8. Use caution when handling patient samples to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
9. Conduct the assay away from bad ambient conditions. e.g. ambient air containing high concentration corrosive gas such as sodium hypochlorite acid, alkaline, acetaldehyde and so on, or containing dust.
10. Do not use reagents beyond the labeled expiry date.
11. Do not mix or use components from kits with different batch codes.
12. Ensure the microparticles are re-suspended before loading it on the analyzer.
13. Avoid foam formation in all reagents and sample types (samples, calibrators and controls).
14. Do not substitute any reagent in this kit from other manufacturers or other lots.
15. When any damage to the protective packaging or any change of analytical performance is observed, do not use the kit.

Storage

1. Store all components of the kit at 2-8 °C. Do not freeze. Avoid strong light.
2. Refrigerate the reagent pack at 2-10 °C for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10°C for a maximum of 28 days. After 28 days, the reagent pack must be discarded. Once they are removed from the analyzer, store them at 2-10 °C in an upright position.
4. Seal and return the remaining calibrators at 2-8 °C immediately after the experiment, under which conditions the stability will be retained for 2 months; for longer use, store opened calibrators in aliquots and freeze at -20 °C. Avoid multiple freeze-thaw cycles.

Sample

1. Collect samples in accordance with correct medical practices.
2. Do not use heat-inactivated samples and samples with obvious microbial contamination. Do not use sodium azide preservative in samples.
3. Sediments and suspended solids in samples may interfere with the test result which should be removed by centrifugation. Ensure that complete clot formation in serum samples has taken place prior to centrifugation. Some samples, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the sample is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results. Be sure that the samples are not decayed prior to use.
4. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
5. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
6. Avoid grossly hemolytic, lipemic or turbid samples.
7. Cap and store the samples at 18-25 °C for no more than 8 hours, for longer use samples should be capped and stored at 2-8 °C up to 24 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20 °C. Avoid multiple freeze-thaw cycles. Mix thawed samples thoroughly by low speed vortex or by inverting 10 times. Visually inspect the samples, if layering or stratification is observed, continue mixing until samples are visibly homogeneous. After thawing, bring to room temperature and mix well by gently shaking.
8. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy in appearance prior to use to ensure consistency in the results.
9. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
10. If proper sample collection and preparation cannot be verified, or if samples have been disrupted due to transportation or sample handling, an additional centrifugation step is recommended. Centrifugation conditions should be sufficient to remove particulate matter.
11. For optimal results, inspect all samples for bubbles. Remove bubbles with a tip prior to analysis. Use a new tip for each sample to prevent cross contamination.

Measurement Procedure

1. Check the consumable materials
 - Verify adequate volume of consumable materials is present prior to running the test.
 - Refer to the Assay Analyzer's operation manual.
2. Load the kit

- Mix contents of new (unpunctured) reagent packs by gently inverting pack several times before loading on the analyzer. Avoid foam formation in all reagents. Don't invert the open (punctured) packs. If necessary, shake gently to mix horizontally after the first loading.
- Read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Refer to the Assay Analyzer's operation manual.

3. Order tests

- Place the sample cups or tubes on the sample rack, 50 µL of samples for each test. But consider the sample container and 150 µL of system dead volumes, which can be refer to the appropriate Assay Analyzer manuals for the minimum sample volume required.
- Load the sample rack and input the sample information on the system software interface.
- Select "run" to start the test, the analyzer automatically operates tests. It performs the following functions:
 - Moves the sample to the set point
 - Loads a reaction vessel into the process path
 - Aspirates and transfers sample into the reaction vessel
 - Adds Microparticles Solution, Enzyme Conjugate and Antibody Solution to the reaction vessel
 - Mixes, incubates and washes the reaction mixture
 - Adds Chemiluminescent Substrate
 - Measures chemiluminescent emission to determine the quantity of PTH in the sample
 - Discards the used reaction vessel
 - Calculates the result
- Refer to the Assay Analyzer's operation manual.

4. Calibrate the curve

- Analyzer can read the bar code on the reagent pack automatically to obtain the required parameters for the test.
- If the bar code cannot be read in exceptional cases, they can be recognized manually.
- Transfer the calibrators into the sample cups or tubes and place them on the sample rack. Conduct duplicate detection on the system.
- Load the sample rack and input calibrators' information on the system software interface.
- Select "run" to start the test and generate the calibration curve, calibration is required every 28 days.
- Once a calibration curve is accepted and stored, all subsequent samples may be tested without further calibration unless:
 - Controls are out of range after repeated measurements
 - A reagent kit and substrate solution with new batch code is used
 - Beyond the expiration date of a calibration curve
 - Important parts of the analyzer are replaced or repaired.
- Refer to the Assay Analyzer's operation manual.

5. Dilute the sample

Samples with a PTH value exceeding 3000pg/mL may be diluted via the program of the analyzer. Diluent Universal is used to dilute the samples. The software takes the dilution into account when reporting the result.

Measurement Results

The sample test results are determined automatically by the system software. The amount of PTH in the samples is determined from the measured light production by means of the stored calibration data. Refer to the Assay Analyzer's operation manual on reviewing the stored data.

Control Procedure

Controls for the various concentration ranges should be run individually when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Limitations of the Procedure

1. This assay is intended as an aid for the clinical diagnosis. Conduct this assay in conjunction with clinical examination, patient's medical history and other test results.
2. If the results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. Heterophilic antibodies and rheumatoid factors in samples may interfere with test results. Heterophilic antibodies in human serum can react with reagent immunoglobulin, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis. This kind of samples is not suitable to be tested by this assay.
4. Patients who have received mouse monoclonal antibodies for either diagnosis or therapy can develop HAMA (human anti-mouse antibodies). HAMA can produce either falsely high or falsely low values in immunoassays which use mouse monoclonal antibodies. Additional information may be required for diagnosis.
5. This test measures concentrations within the range of 2pg/mL - 3000pg/mL. If PTH concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal, the recommended dilution is 1:9 of this test, under this condition, allowing samples to be up to approximately 30000pg/mL.

Biological Reference Interval

A normal range of 11.0pg/mL to 81.0pg/mL (95% confidence interval) was obtained by testing serum samples from 148 individuals defined as normal by clinician. It is recommended that each laboratory establish its own normal range which may be unique to the population it serves depending upon geographical, patient, dietary, or environmental factors.

Performance Characteristics

1. Measurement Precision

3 samples were assayed in duplicate, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean (pg/mL)	Within-run	Total
			%CV	%CV
1	80	22.23	3.83	5.73
2	80	219.27	2.63	4.73
3	80	938.90	3.67	4.79

*Representative data; results in individual laboratories may vary from these data

2. Analytical Sensitivity

Limit of Blank: 2.0pg/mL.

Limit of Detection: 4.0pg/mL.

Limit of Quantitation: 8.0pg/mL with a coefficient of variation of $\leq 20\%$.

3. Analytical Specificity

Cross reaction: No cross reaction with following substances at listed concentration levels.

Substance	Concentration pg/mL	Measured Value pg/mL
PTH (1-34)	10000	≤ 2.0
PTH (39-84)	10000	≤ 2.0

Interference: No interference with 20 mg/dL of bilirubin, 200 mg/dL of hemoglobin, 3000 mg/dL of triglycerides.

4. Measurement Accuracy by Correlation

A study was performed where samples were tested using this assay and PTH Test which was already available on the market. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	214	0.9595	-0.4799	0.9939

5. High Dose Hook Effect

A sample spiked with PTH up to 500000pg/mL was determined, the concentration result obtained was ≥ 3000 pg/mL.

Literature References

1. Bieglmayer C, Prager G, Niederle B (October 2002). "Kinetic analyses of parathyroid hormone clearance as measured by three rapid immunoassays during parathyroidectomy". Clin. Chem. 48 (10): 1731-8. PMID 12324490.
2. Prahald AK, Hickey RJ, Huang J, et al. (June 2006). "Serum proteome profiles identifies parathyroid hormone physiologic response". Proteomics 6 (12): 3482-93. doi:10.1002/pmic.200500929. PMID 16705755.
3. Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH (May 2001). "Effect of parathyroid hormone (1-34) on fractures and bone mineral density in postmenopausal women with osteoporosis". N. Engl. J. Med. 344 (19): 1434-41. doi:10.1056/NEJM200105103441904. PMID 11346808.