MAGLUMI intact PTH (CLIA)

1. INTENDED USE
The kit has been designed for the quantitative determination of intact Parathyroid hormone (intact PTH) in human serum. The method can be used for samples over the range of 0-5000pg/ml. The test has to be performed on the Maglumi fully auto analyzer (including Maglumi 1000, Maglumi 2000, Maglumi 2000 plus).

2. SUMMARY AND EXPLANATION OF THE TEST
PTH (parathyroid), a single-chain polypeptide (with a molecular mass of approximately 9,500 daltons) containing 84 amino acids, exerts significant influence in the maintenance of optimal calcium ion concentrations. PTH raises serum ionized calcium levels through direct action on bone and the kidneys: it increases the rate of calcium ion flow from bone to the extracellular fluid, and increases both the renal tubular reabsorption of ionized calcium and the renal excretion of phosphate. Long-term regulation of total body calcium by PTH occurs through its stimulation of vitamin D metabolism, which results in enhanced intestinal absorption of ionized calcium.

In healthy individuals, PTH is secreted in response to circulating calcium ion levels. Any dip below an individual’s normal level triggers a pronounced increase in PTH secretion. Calcium levels returning to normal exert a negative feedback effect, thus inhibiting PTH secretion by the parathyroid glands.

PTH undergoes proteolysis to a lesser extent in the parathyroid glands but mostly peripherally – especially in the liver but also in the kidneys and bone – to yield N-terminal fragments and longer lived C-terminal and midregion fragments. The N-terminal fragment contains the region that confers bioactivity. C-terminal and N-terminal fragments are initially generated in equivalent amounts, but the N-terminal fragments disappear rapidly. The C-terminal fragment has a half-life of several hours. In renal failure, C-terminal fragment clearance by glomerular filtration is impaired, so that high levels are found. C-terminal assays (as well as midregion assays) are consequently likely to be especially unreliable in chronic renal failure, where increased PTH is typically just a reflection of impaired renal clearance.2

For the intact hormone, the in vivo half-life is 2 to 5 minutes. Intact PTH clearance is accomplished by both peritubular uptake and glomerular filtration followed by reabsorption. In normal renal function, intact PTH is the greatest part of circulating PTH-like bioactivity4 and is present in the circulation at concentrations of 10-11 to 10-12 mмол. In hypercalcemia due to primary hyperparathyroidism or to ectopic PTH production (psuedohyperparathyroidism), the majority of patients have elevated PTH levels. By contrast, in hypercalcemia due to malignancy or other causes, the concentration of PTH in circulation is typically low or within normal reference range limits. PTH levels are also characteristically high in secondary hyperparathyroidism – usually associated with renal failure – as a result of constant stimulation of the parathyroid gland by low calcium levels. Hypocalcemia accompanied by a low PTH level, on the other hand, is to be expected in hyperparathyroidism, either postsurgical or idiopathic.

In vitro diagnostic medical device (In vitro diagnostic use)

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3. PRINCIPLE OF THE TEST
Sandwich immunoluminometric assay;
Use an anti-PTH monoclonal antibody to label ABEI, and use another monoclonal antibody to label FITC. Sample, Calibrator, or Control, ABEI Label, FITC Label and magnetic microbeads coated with anti-FITC are mixed thoroughly and incubated at 37°C, forming a sandwich; after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is initiated. The light signal is measured by a...
4. KIT COMPONENTS
4.1 Material supplies

<table>
<thead>
<tr>
<th>Reagent Integral for 100 determinations</th>
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<tbody>
<tr>
<td>Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaCl, coated with sheep anti-FITC polyclonal antibody.</td>
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<tr>
<td>Calibrator: low.</td>
</tr>
<tr>
<td>Calibrator: high.</td>
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<tr>
<td>ABEI Label: anti-PTH monoclonal antibody labeled ABEI contains BSA, 0.2%NaCl.</td>
</tr>
<tr>
<td>FITC Label: anti-PTH monoclonal antibody labeled FITC, contains BSA, 0.2%NaCl.</td>
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<tr>
<td>All reagents are provided ready-to-use.</td>
</tr>
</tbody>
</table>

4.2 Preparation of the Reagent Integral

Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation()! Remove the sealing and turn the small wheel of the magnetic microbeads compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 mins. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

**Do not interchange nano magnetic microbeads from different reagents.**

4.3 Storage of the Reagents Integral

- Sealed: Stored at 2-8°C until the expiry date.
- Opened: Stable for 4 weeks. After this period, it is still possible to keep on using the Reagent Integral provided that the controls are found within the expected ranges.
- Keep upright for storage.
- Keep away from direct sunlight.

5. Origin of Calibrators

Calibrators in the reagent kit are from Sigma.

6. Calibration

6.1 2 point recalibration

Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each calibration.

6.2 Frequency of Recalibration

- After each exchange of lot (Reagent Integral or Starter Reagents).
- After 4 weeks and/or each time a new Integral is used (recommendation).
- After each servicing of the Maglumi Fully Auto analyzer.
- If controls are beyond the expected range.

7. Sample Collection, Material and Storage

- Collect samples using standard procedures.
- Sample material: serum.
- Store at 2-8°C: 24 hours.
- For longer storage periods: freeze to below -20°C.
- Avoid repeated freezing and thawing cycles.
- Stored samples should be thoroughly mixed prior to use (Vortex mixer).
- **Vacuum tubes**
  - (a) Blank tubes are recommended type for collecting samples.
  - (b) If plasma sample is needed, EDTA tube is conformable has no effect on the results RUls.
  - (c) Liquaemin Sodium tube is found to increase the sample RUl and cause test results deviation.
  - (d) Please ask SNIBE for advice if special additive must be used in the sample blood.

8. WARNING AND PRECAUTIONS FOR USERS

- For use in IN-VITRO-diagnostic procedures only.
- Do not interchange reagents from different lots. Do not use kit components beyond their labeled expiry date.
- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an overkill approach (USP 24.2000, p.2143). A minimum of one hour at 110°C is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.

- The calibrators in this kit are prepared from bovine serum products. However, because no test method can offer complete assurance that HIV, Hepatitis B Virus or other infectious agents are absent, these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.

9. Test Procedure

To ensure proper test performance, strictly adhere to the operating instructions of the Maglumi Fully Auto analyzer. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

<table>
<thead>
<tr>
<th>100μl</th>
<th>Sample, calibrator or controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>50μl</td>
<td>ABEI Label</td>
</tr>
<tr>
<td>50μl</td>
<td>FITC Label</td>
</tr>
<tr>
<td>250μl</td>
<td>Nano magnetic microbeads</td>
</tr>
</tbody>
</table>

30 min Incubation

400μl each time Cycle washing

3 s Measurement

10. Quality Control

- Observe quality control guidelines for medical laboratories.
- Use suitable controls for in-house quality control.

11 Results

11.1 Calculation of Results

- The analyzer automatically calculates the intact PTH concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The result is expressed in pg/ml. For further information please refer to the Maglumi Fully Auto Operator’s Manual.
- For the PTH lots of the year 2013 please use the correction factor 0.28 as a conversion factor (do not omit to convert the control target value and range values).
- Reference values are not expected to be changed.

11.2 Interpretation of Results

- Reference values: <30pg/ml.
- Results may differ between laboratories due to variations in population and test method. Each laboratory should establish its own reference range.

12. Limitations of the procedure

12.1 A skillful technique and strict adherence to the instructions are necessary to obtain reliable results. Bacterial contamination of samples or repeated freeze-thaw cycles may affect the test results. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.

12.2 HAMA

Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralising agents are added, extremely high HAMA serum concentrations may occasionally influence results.

12.3 High-Dose Hook

No high-dose hook effect was seen for intact PTH concentrations up to 10 ng/ml. If RUl value of the samples is higher than Calibrator high on the curve, the concentration calculated by the instrument is not necessarily accurate. For these samples, it is recommended to dilute them until an RUl value ranging between Standard A and calibrator high is exhibited. After that, send them for a measurement. The output value multiplied by dilution ratio equals the final RUl value of samples.

13. Performance Characteristics

13.1 Accuracy

Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratio with diluent, and measure its diluted concentration for 10 times. Then calculate the recovery of measured concentration and expected concentration. The recovery should be within 90%-110%.

13.2 Precision

Intra-assay coefficient of variation was evaluated on Calibrator High
repeatedly measured 10 times in the same assay, calculating their coefficient of variation, the results should ≤10%.

Inter-assay coefficient of variation was evaluated on three batches of kit, repeatedly measured 10 times of Calibrator High, calculating three batches of kit for Calibrator High between the measured values of the coefficients of variation, the results should ≤15%.

13.3 Sensitivity
The sensitivity is defined as the concentration of PTH equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 12.5 pg/ml.

13.4 Specificity
The specificity of the PTH assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes. When ACTH = 100 pg/ml, the detection results of PTH < 1 pg/ml.

13.5 Linearity
Conduct a logarithmic transform to the RLU value and concentration value of 6 Calibrators. After a double logarithmic fitting, the absolute value of its linearity should exceed 0.9800.

14. References