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Immunoassay

REF CML0201 / CML0202 / CML0203 / CML0204 / CML0205

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

HGH CLIA Microparticles

This assay is based on a chemiluminescent microparticle immunoassay(CLIA Microparticles) for the quantitative determination of HGH (Human Growth Hormone)concentration in human serum and plasma (Heparin).

Key to Graphical Symbols Used

LOT	batch code		use by
	manufacturer	Σ	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		

EC REP

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Introduction

Human Growth Hormone (HGH) is a peptide hormone that stimulates growth, cell reproduction and regeneration in humans and other animals. Growth hormone is a single-chain polypeptide that is synthesized, stored, and secreted by somatotropin cells within the lateral wings of the anterior pituitary gland. Human Growth Hormone is a stress hormonethat raises the concentration of glucose and free fatty acids[1,2]. It also stimulates production of IGF-1.

The major isoform of the human growth hormone is a protein of 191amino acids and a molecular weight of 22,124 daltons[3]. Effects of HGH on the tissues of the body can generally be described as anabolic (building up). Like most other protein hormones, HGH acts by interacting with a specific receptor on the surface of cells. Increased height during childhood is the most widely known effect of growth hormone[4].

Excessive secretion of hormones in kids is a reason for increase in their height. As age increases, the secretion reduces and this directly results in certain body related issues. The medicines increases energy, reduces body fat, makes heart resistant to diseases, bones becomes stronger, rejuvenates skin, improves memory, develops immune system further and there are many more benefits of HGH[5].

Growth hormone is essential for children to grow normally. The role of the growth hormone in adults is to maintaining the necessary levels of body fat, muscle and bone. Inadequate or no growth hormone in adults leads to emotional problems like tiredness and lack of motivation, and sometimes affects cholesterol level as well[4].Deficiency in growth hormone occurs due to inadequate or absence of secretion of growth hormone. The conditions responsible for this may either be congenital which occurs from birth or acquired which results after birth. The cause of congenital growth hormone deficiency could be due to an abnormal pituitary gland or another syndrome altogether[6].

Measurement Principle

This assay is based upon the one-step sandwich method. The sample, anti-HGH coated microparticles and enzyme labeled anti-HGH are added to the reaction vessel. During incubation, HGH present in the sample is allowed to react simultaneously with the two antibodies, resulting in the HGH being sandwiched between the microparticles-coated antibodies and enzyme-labeled antibodies. After washing, a complex is generated among the solid phase, the HGH within the sample and enzyme-linked antibodies by immunological reactions. Chemiluminescent Substrate is then added and catalyzed by this complex, resulting in a chemiluminescent reaction. The resulting chemiluminescent reaction is measured as RLUs. The RLU is proportional to the amount of HGH in the samples.

Materials Provided

1. Calibrators

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

2. Reagent pack

Reagent pack provided ready to use.

Σ	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	5.5mL*1	11.0mL*1	11.0mL*2	11.0mL*5	5.5mL*2

- Microparticles Solution

Contains of mouse monoclonal anti-HGH coated microparticles in PBS (phosphate buffered saline) buffer containing BSA. Contains a selection of preservatives.

- Enzyme Conjugate

Contains of horseradish-peroxidase labeled mouse monoclonal anti-HGH in Tris-NaCl buffer containing BSA(bovine serum albumin). 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 Analyzers which are AutoLumo A2000 Plus, AutoLumo A2000 Plus B and AutoLumo A1000.

Materials Required but not Provided

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

Metrological Traceability of Calibrators

The analyte in these HGH calibrators is traceable to a calibrator purchased from NICPBP (National Institute for the Control of Pharmaceutical and Biological Products), China, at each concentration level.

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 instruction for use.
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. Some reagents contain ProClin 300®may cause sensitization by skin-contact, which must be avoided to contact with skin. This material and its container must be disposed in a safe way. If swallowed, seek medical advice immediately.
7. Do not smoke, drink, eat or use cosmetics in the working area.
8. Wear protective clothing and disposable gloves when dealing with samples and reagents. Wash hands after operations.
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 mix or use components from kits with different batch codes.
11. When storing the calibrators, be certain the vials are securely sealed.
12. Ensure the microparticles are resuspended 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.
16. Do not use reagents beyond the labeled expiry date.

Storage

1. Store the kit at 2-8℃. Do not freeze. Avoid strong light.
2. Refrigerate the reagent pack at 2-10 ℃ for a minimum of 2 hours prior to use.
3. Store the unsealed reagents pack upright on the analyzer or 2-10℃ 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 ℃ in an upright position.
4. Seal and return the remaining calibrators at 2-8℃ immediately after the experiment, under which conditions the stability will be retained for 2 months.

Sample

1. Collect serum or plasma samples in accordance with correct medical practices (Citrate and EDTA anticoagulants are not recommended).
2. Do not use heat-inactivated samples. Do not use sodium azide preservative in samples.
3. Do not use samples with obvious microbial contamination.
4. 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.
5. Prior to shipment, it is recommended that samples be removed from the clot, serum separator or red blood cells.
6. Insufficient processing of sample or disruption of the sample during transportation may cause depressed results.
7. Avoid grossly hemolytic, lipemic or turbid samples.
8. Cap and store the samples at 18-25 ℃ for no more than 8 hours, for longer use samples should be capped and stored at 2-8℃up to 48 hours. Or freeze the samples that need to be stored or transported for more than 48 hours at -20℃. 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.
9. Centrifuge the thawed samples containing red blood cells or particulate matter, or which are hazy or cloudy etc. in appearance prior touse to ensure consistency in the results.
10. Note that interfering levels of fibrin may be present in samples that do not have obvious or visible particulate matter.
11. 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.
12. 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 tube(s) or cup(s) on the sample rack,25 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 Microparticle Solution and Enzyme Conjugate to the reaction vessel
  - Mixes, incubates and washes the reaction mixture
  - Adds Chemiluminescent Substrate
  - Measures chemiluminescent emission to determine the quantity of HGH 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 tube(s) or cup(s) 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 every28 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 Chemiluminescent Substrate 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 HGH value exceeding 50 ng/mL may be diluted with the program of the analyzer. Diluent Universal is used to dilute the samples. The software automatically 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 HGH in the samples is determined from the measured light production by means of the stored calibration data. Sample test results can be reviewed using the appropriate computer or printed out. Refer to the Assay Analyzer's operation manual on reviewing sample results.
The default unit for this assay is ng/mL.

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 immunoglobulins, 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. Because of pulsatile secretion, samples obtained within the same day from the same patient may fluctuate widely within the reference interval, reflecting physiological variation rather than errors in technique or methodology.
5. This assay was designed and validated for use with human serum from individual patient and donor samples. Pooled samples must not be used since the accuracy of their test results has not been validated.
6. This test measures concentrations within the range of 0.02-50 ng/mL. If HGH concentrations above the measuring range to be expected, it is recommended to dilute samples with Diluent Universal. The recommended dilution is 1:3 of this test, allowing samples to be up to approximately 200 ng /mL.

Biological Reference Interval

The suggested normal range (95% confidence interval) was obtained by testing serum samples from 102 normal adult males and 110 normal adult females. The results are presented in the following table. 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.

Sample type	N	Mean Value (ng/mL)	Reference Interval (ng/mL)
Males	102	0.599	0.02-1.506
Female	110	3.171	0.024-5.419

Performance Characteristics

1. Measurement Precision

3 samples were assayed in duplicate of 2, twice per day across 20 testing days. Data from this study are summarized in the following table.

Sample	n	Mean (ng/mL)	Within-run	Total
			%CV	%CV
1	80	1.38	4.05	6.10
2	80	4.52	4.50	6.18
3	80	21.14	4.45	6.57

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

2. Analytical Sensitivity

Limit of Blank: 0.002 ng/mL.
Limit of Detection: 0.002 ng/mL.
Limit of Quantitation: 0.003 ng/mL with a coefficient of variation of 20%.

3. Analytical Specificity

Cross reaction: the following substances and concentrations were tested and found no cross reaction with the test.

Substances	Concentration
FSH	100 IU/L
TSH	100 mIU/L
LH	100 IU/L
PRL	4000 mIU/L
HCG	2280 IU/L

Interference: No interference with 20 mg/dL of Bilirubin, 200 mg/dL of Hemoglobin, 3000 mg/dL of Triglyceride.

4. Measurement Accuracy by Correlation

A comparison study was performed where samples were tested using this assay and an HGH reference assay. Data were analyzed and are summarized in the following table.

Correlation Method	Number of Samples	Intercept	Slope	Correlation Coefficient
Linear Regression	82	-0.331	0.98	0.9935

5. High Dose HooK Effect

A sample spiked with HGH up to 1000 ng/mLwas determined, the concentration result obtained was ≥50 ng/mL.

Literature References

1.Ranabir S& Reetu K. 2011. Stress and hormones. Indian J Endocrinol Metab.15 (1): 18–22.

2. Greenwood FC& Landon J.1966. Growth hormone secretion in response to stress in man. Nature. 210 (5035): 540–1.

3.Leung KC, Howe C, Gui LY, Trout G, Veldhuis JD and Ho KK. 2002. Physiological and pharmacological regulation of 20-kDa growth hormone. Am. J. Physiol. Endocrinol. Metab. 283 (4): E836–43.

4. Nyberg F& Hallberg M. 2013. Growth hormone and cognitive function.Nat Rev Endocrinol. 9 (6): 357–65.

5.Nørrelund H.2005. The metabolic role of growth hormone in humans with particular reference to fasting. Growth Horm. IGF Res. 15 (2): 95–122.

6.Prodam F, Caputo M, Belcastro S, Garbaccio V, Zavattaro M, Samà MT, Bellone S, Pagano L, Bona G and Aimaretti G. 2012. Quality of life, mood disturbances and psychological parameters in adult patients with GH deficiency. Panminerva Med. 54 (4): 323–31.