DiaSorin Italia S.p.A.
Via Crescentino snc - 13040 Saluggia (VC) - Italy
www.diasorin.com
Tel. +39.0161.4871

Changes: §13;
Deletions: -

LIAISON® hGH (REF 310340)

1. INTENDED USE

The LIAISON® hGH assay uses chemiluminescence immunoassay (CLIA) technology for the *in vitro* quantitative determination of human growth hormone in human serum specimens.
The test has to be performed on the LIAISON® Analyzer family.*

2. SUMMARY AND EXPLANATION OF THE TEST

Human growth hormone (hGH or somatotropin) is the hormone most abundantly secreted by the pituitary. During childhood and adolescence it causes body statural growth, and throughout life it affects major metabolic processes, in such a way that growth is encouraged.
hGH is synthesized by the alpha cells of the anterior pituitary. It is composed of a single chain of 191 amino acid residues with a molecular weight of approximately 22 kDa and two disulphide bonds.
hGH circulates in different molecular forms, called Big-Big GH, Big GH and Little GH. Little GH is the most abundant form and probably the original monomeric form, while the other two seem to be polymeric forms. The major monomeric form is the 22-kDa isoform (90% of hGH synthesized) and the second most abundant form is the 20-kDa variant (5-10% of hGH synthesized), produced by alternative splicing of hGH messenger ribonucleic acid (mRNA).
hGH secretion is controlled by the hypothalamic growth hormone-releasing hormone (GH-RH) and somatostatin, which stimulate and inhibit release, respectively.

hGH is secreted episodically (pulsatile secretion) and, in normal subjects, it is modulated by many factors including stress, physical exercise, and sleep. Because of marked fluctuations in daily secretion, a single random specimen has limited clinical interest. Challenge tests (with arginin, insulin, glucagon, L-dopa, GH-RH) or inhibition tests (glucose tolerance test) give more detailed information.
hGH does not have a specific target organ, but receptors have been demonstrated on human liver cells and circulating lymphocytes. The metabolic effects of hGH can be generally divided into two main categories: *(a)* anti-insulin direct action (lipolytic and causing hyperglycaemia); *(b)* insulin-like indirect action (anti-lipolytic and mitogenic). The latter action causes body statural growth which is the classic action of hGH. This is exerted through somatomedins, relatively low-molecular weight hormones (M.W. 7 kDa) of chemical structure very similar to that of proinsulin.
At least two somatomedins have been identified, one with insulin-like properties, the other one more directly involved in body statural growth. Unlike all other polypeptide hormones, somatomedins mostly circulate bound to carrier proteins which prolong their half-lives, therefore making their concentrations relatively stable.

The test is of help in correct diagnosis of growth disorders, namely:
– Hormone deficiency, including delayed puberty and small stature in adolescents.
– Hormone excess, associated with gigantism in young children or adolescents or acromegaly in adults.
– In addition, the test may be of use to evaluate growth hormone replacement therapy.

3. PRINCIPLE OF THE PROCEDURE

The method for the quantitative determination of hGH is a sandwich chemiluminescence immunoassay.
A specific mouse monoclonal antibody to hGH is coated on the magnetic particles (solid phase); another monoclonal antibody (specific for a different epitope of the hGH molecule) is linked to an isoluminol derivative (isoluminol-antibody conjugate).
During the incubation, hGH present in calibrators, samples or controls binds to the solid phase monoclonal antibody, and subsequently the antibody conjugate reacts with hGH already bound to the solid phase. A sandwich is formed only in the presence of hGH molecules that bridge both antibodies. After incubation, the unbound material is removed with a wash cycle.
Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is directly proportional to hGH concentration present in calibrators, samples or controls.

*(LIAISON®, LIAISON® XL, LIAISON® XS)

4. MATERIALS PROVIDED

The order of reagents reflects the layout of containers in the reagent integral.

Reagent Integral for 100 determinations		
Magnetic particles (2.3 mL)	SORB	Magnetic particles (suspension) coated with anti-hGH monoclonal antibody (mouse), bovine serum albumin, PBS buffer, < 0.1% sodium azide.
Conjugate (13 mL)	CONJ	Anti-hGH monoclonal antibody (mouse), labelled with isoluminol derivative, sheep serum, non-specific mouse IgG, bovine serum albumin, PBS buffer, 0.2% ProClin™ 300, preservatives.

Included in the kit:

Calibrator A (1.5 mL)	CAL A	Recombinant hGH (obtained in *E. coli*), human hGH-free serum, phosphate buffer, 0.2% ProClin™ 300, preservatives. (lyophilized reagent)
Calibrator B (1.5 mL)	CAL B	Recombinant hGH (obtained in *E. coli*), human hGH-free serum, phosphate buffer, 0.2% ProClin™ 300, preservatives. (lyophilized reagent)
2 Bar-coded labels for calibrator A and for calibrator B.		

Conjugate and magnetic particles are provided ready-to-use. Calibrators are provided lyophilized.

Materials required but not provided (system related)

LIAISON® XL Analyzer	**LIAISON® Analyzer**
LIAISON® XL Cuvettes (REF X0016). LIAISON® XL Disposable Tips (REF X0015) or LIAISON® Disposable Tips (REF X0055). LIAISON® XL Starter Kit (REF 319200) or LIAISON® EASY Starter Kit (REF 319300) LIAISON® Wash/System Liquid (REF 319100). LIAISON® XL Waste Bags (REF X0025).	LIAISON® Module (REF 319130). LIAISON® Starter Kit (REF 319102) or LIAISON® XL Starter Kit (REF 319200) or LIAISON® EASY Starter Kit (REF 319300) LIAISON® Light Check 12 (REF 319150). LIAISON® Wash/System Liquid (REF 319100). LIAISON® Waste Bags (REF 450003). LIAISON® Cleaning Kit (REF 310990).

LIAISON® XS Analyzer
LIAISON® Cuvettes on Tray (REF X0053). LIAISON® Disposable Tips (REF X0055). LIAISON® EASY Starter Kit (REF 319300). LIAISON® EASY Wash Buffer (REF 319301). LIAISON® EASY System Liquid (REF 319302). LIAISON® EASY Waste (REF X0054). LIAISON® EASY Cleaning Tool (REF 310996).

Additionally required materials

LIAISON® hGH controls, levels 1 and 2 (REF 310341).
LIAISON® Endocrinology Diluent (REF 319133).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

All materials used to produce the components provided in this kit have been tested for the presence of HBsAg, anti-HCV, anti-HIV-1, anti-HIV-2 and found to be non-reactive. As, however, no test method can offer absolute assurance that pathogens are absent, all specimens of human origin should be considered potentially infectious and handled with care.

6. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics in the assay laboratory.

Do not pipette by mouth.

Avoid direct contact with potentially infected material by wearing laboratory clothing, protective goggles, and disposable gloves. Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infected waste.

All samples and reagents containing biological materials used for the assay must be considered as potentially able to transmit infectious agents. The waste must be handled with care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each Country. Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle.

Do not use kits or components beyond the expiration date given on the label.

Pursuant to EC Regulation 1272/2008 (CLP), hazardous reagents are classified and labeled as follow:

REAGENTS:	CONJ	CAL A (lyophilized), CAL B (lyophilized)
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412	Eye irrit. 2 H319 Skin irrit. 2 H315 Skin sens. 1A H317 Aquatic Acute 1 H400 Aquatic Chronic 1 H410
SIGNAL WORD:	Warning	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 – Exclamation mark	  GHS07 Exclamation mark GHS09 Environment
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.	H315 Causes skin irritation. H317 May cause an allergic skin reaction. H319 Causes serious eye irritation. H410 Very toxic to aquatic life with long lasting effects.
PRECAUTIONARY STATEMENTS:	P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash before reuse.	P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P273 avoid release to the environment. P391 Collect spillage.
CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008).	reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin™ 300).	reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin™ 300); gentamycin sulfate salt.

Pursuant to EC Regulation 1272/2008 (CLP), after reconstitution CAL A and CAL B are classified and labeled as follow:

REAGENTS:	CAL A (reconstituted), CAL B (reconstituted)
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412
SIGNAL WORD:	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 – Exclamation mark
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.
PRECAUTIONARY STATEMENTS:	P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash before reuse.
CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008).	reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin™ 300).

Pursuant to EC Regulation 1272/2008 (CLP), SORB is labeled as EUH210 safety data sheets available on request.
For additional information see Safety Data Sheets available on www.diasorin.com.

7. REAGENT PREPARATION

REAGENT INTEGRAL

Please note the following important reagent handling precautions:

Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension:
Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the colour of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have resuspended. Repeat as necessary until the magnetic particles are completely resuspended. After removal of the seal carefully wipe the surface of each septum to remove residual liquid if necessary.

Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:
Visually inspect the reagents to ensure there is no foaming present before using the integral. If foam is present after resuspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

Loading of integral into the reagent area

LIAISON® Analyzer

– Place the integral into the reagent area of the analyzer with the bar code label facing left and let it stand for 30 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
– Follow the analyzer operator's manual to load the specimens and start the run.

LIAISON® XL and LIAISON® XS Analyzers

– LIAISON® XL and LIAISON® XS analyzers are equipped with a built-in solid-state magnetic device which aids in the dispersal of microparticles prior to placement of a reagent integral into the reagent area of the analyzer. Refer to the analyzer operator's manual for details.
  a. Insert the reagent integral into the dedicated slot.
  b. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.
– Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
– Follow the analyzer operator's manual to load the specimens and start the run.

CALIBRATORS

LIAISON® hGH calibrators are supplied lyophilized.

– Reconstitute the vial contents with 1.5 mL deionized or distilled water.
– Allow the vials to stand for 10-15 minutes at 18-25°C to achieve complete dissolution.
– Mix vials thoroughly by gentle inversion; avoid foaming.
– The reconstituted solution of each calibrator must be divided into two 750-µL aliquots and transferred to 12 x 75 mm polystyrene tubes. Affix the proper bar-coded labels to the calibrator tubes and load on to the instrument. Each calibrator solution allows at least five calibrations to be performed.

Once reconstituted refer to paragraph 8 to store the calibrators.
For details on the use of the calibrators on board the instrument, refer to the relevant LIAISON® Operator's Manual.

Vials labels refer only to lyophilized calibrators. Once reconstituted, pursuant to EC Regulation 1272/2008 (CLP), calibrators are classified Skin sens. 1A H317 - Aquatic chronic 3 H412. For more details refer to paragraph 6.

CONTROLS

Refer to the LIAISON® hGH Control Set instructions for use section for proper preparation and handling instructions.

8. REAGENT STORAGE AND STABILITY

REAGENT INTEGRAL

– **Sealed**: Stable at 2-8°C until the expiry date.
– **Opened on board or at 2-8°C**: Minimum stability four 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.
– Use always the same analyzer for a reagent integral already opened.
– Use storage rack provided with the analyzer for upright storage of reagent integral.
– Do not freeze.
– Keep upright for storage to facilitate later proper resuspension of magnetic particles.
– Keep away from direct light.

CALIBRATORS

– **Lyophilized**: Stable at 2-8°C until the expiry date. Upon receipt, the calibrators must be stored at 2-8°C in an upright position to prevent adherence of the lyophil to the vial cap.
– **Reconstituted**: Stable for four weeks when properly stored at 2-8°C either in their sealed vials or in stoppered transfer tubes. After reconstitution the calibrators must be stored at 2-8°C in an upright position to prevent adherence of the solution to the vial or tube cap. For extended storage, deep-freeze at –20°C or below.

Do not leave the reconstituted calibrators at room temperature longer than the time required to process them on the analyzer.
During handling, use appropriate precautions to avoid bacterial contamination of calibrators.

9. SPECIMEN COLLECTION AND PREPARATION

The only sample material validated is human serum. Use of plasma samples was not evaluated and is therefore not recommended.
Collect blood by venipuncture in tubes containing no additives and allow the blood to clot according to the laboratory procedures, ensuring sample integrity is maintained. Separate serum from the clot as soon as possible. The presence of haemolysis may indicate mistreating during sample collection or handling. If the assay is performed within 48 hours of sample collection, the samples should be kept at 2-8°C; otherwise they should be aliquoted and stored deep-frozen (–20°C or below). Sixteen samples underwent five freeze-thaw cycles. The results showed no significant differences.

Carefully thaw before testing, mix the thawed samples and check for and remove air bubbles before assaying.
Grossly haemolyzed or lipaemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested.
Do not use clotted samples.
It is recommended to test serum samples immediately after loading on to the instrument.
The minimum volume required for a single determination is 180 µL specimen (30 µL specimen + 150 µL dead volume).

10. CALIBRATION

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the assigned master curve. Each calibration solution allows five calibrations to be performed.

Calibrators must be used only with the reagent integral lot they are matched with. Do not use calibrators matched with a different reagent integral lot in the same assay. For correct lot matching, calibrator lot number is printed also on the reagent integral label.

Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:
– A new lot of Starter Kit is used.
– The previous calibration was performed more than one week before.
– Each time a new lot of integral is used.
– **LIAISON® and LIAISON® XL analyzers:** the Analyzer has been serviced.
– **LIAISON® XS Analyzer**: after a technical intervention, only if required by the service procedure, as communicated by local DiaSorin technical support or representative.
– Control values lie outside the expected ranges.

LIAISON® Analyzer: Calibrator values are stored in the reagent integral bar codes.

LIAISON® XL Analyzer: Calibrator values are stored in the reagent integral Radio Frequency IDentification transponder (RFID Tag).
LIAISON® XS Analyzer: Calibrator values are stored in the reagent integral Radio Frequency IDentification transponder (RFID Tag).

11. ASSAY PROCEDURE

LIAISON® Analyzer. Each test parameter is identified via the bar codes on the reagent integral label. In the event that the barcode label cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.

LIAISON® XL and LIAISON® XS Analyzers. Each test parameter is identified via information encoded in the reagent integral Radio Frequency IDentification transponder (RFID Tag). In the event that the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.

In case external calibrator bar codes fail to be read, data present on the external calibrator labels (under the bar code) may be manually entered on the LIAISON® Analyzer family. For details, refer to the relevant analyzer operator's manual.

The analyzer operations are as follows:
1. Dispense calibrators, controls or specimens into the reaction module.
2. Dispense coated magnetic particles.
3. Dispense conjugate into the reaction module.
4. Incubate.
5. Wash with Wash/System liquid.
6. Add the Starter Kit and measure the light emitted.

12. QUALITY CONTROL

LIAISON® controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® hGH controls
(a) at least once per day of use,
(b) whenever a new reagent integral is used,
(c) whenever the kit is calibrated,
(d) whenever a new lot of Starter Reagents is used,
(e) to assess adequacy of performance of the open integral beyond four weeks, or in agreement with guidelines or requirements of local regulations or accredited organizations.

Control values must lie within the expected ranges: whenever one or both controls lie outside the expected ranges, calibration should be repeated and controls retested. If control values obtained after successful calibration lie repeatedly outside the predefined ranges, the test should be repeated using an unopened control vial. If control values lie outside the expected ranges, patient results must not be reported.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should then be established for quality control materials used.

13. INTERPRETATION OF RESULTS

The Analyzer automatically calculates hGH concentrations for the unknown samples. For details, refer to the Analyzer Operator's Manual.
Calibrators and controls may give different RLU or dose results on LIAISON®, LIAISON® XL and LIAISON® XS but patient results are equivalent.

Assay range: The Analyzer directly calculates hGH concentration up to 80 ng/mL (240 µIU/mL).

Reference standard: The assay is referenced to the World Health Organization Second International Standard 98/574 for Somatotropin (22-kDa recombinant DNA-derived materials). The results are expressed as ng/mL.
To convert ng/mL to µIU/mL multiply results by a factor 3.0.

Expected values: Each laboratory should establish its own range of expected values for the population taken into consideration.
hGH is secreted by the anterior pituitary gland following a pulsatile, episodic rhythm. The secretion is influenced by time of day, physical exercise, sleep, stress, nutritional status, and is regulated by a number of factors like growth hormone-releasing hormone, insulin, glucose, insulin-like growth factor I (IGF-I). For those reasons, no single hGH determination provides useful diagnostic information. Even among apparently healthy individuals, a single result is of no help. To assess the clinical status of patients affected by hGH secretion disorders, challenge tests are commonly used to stimulate (or suppress) hGH release, with a baseline (sample taken before the stimulus) and post-stimulation serial blood sampling. Definition of subnormal response to stimulation varies in accordance to the laboratory.
A study performed in 214 blood donor samples (99 adult males and 115 adult females) yielded the following results. These results refer to the groups of samples investigated and are not guaranteed specifications, but are indicative only.

Subjects	Number of subjects	Mean value (ng/mL)	Median value (ng/mL)	5th-95th percentile (ng/mL)
Total population	214	0.96	0.21	<0.05-4.77
Apparently healthy adult females	115	1.57	0.75	0.06-6.88
Apparently healthy adult males	99	0.25	0.05	<0.05-1.23

14. LIMITATIONS OF THE PROCEDURE

- The reagents should be used only in the LIAISON® System.
- Calibrators are kit lot specific and must not be interchanged with a reagent integral from a different lot.
- Single components of the reagent integral should not be removed from the integral.
- This kit must not be used after the expiry date printed on the package label.
- A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
- Bacterial contamination or heat inactivation of the specimens may affect the test results.
- A result within the expected range does not rule out the presence of disease and should be interpreted together with the patient's clinical picture and other diagnostic procedures.
- Test results are reported quantitatively. However, diagnosis of a disease should not be based on the result of a single test, but should be determined in conjunction with clinical findings in association with medical judgement. Any therapeutical decision must also be taken on a case-by-case basis.
- The LIAISON® hGH assay has been developed for the determination of the analyte in its intact and unaltered state. Degradation of the molecule may affect final results.
- Although HAMA-neutralizing agents are added, extremely high HAMA (human anti-mouse antibodies) concentrations may occasionally influence results.
- No interference due to drug administration has been investigated.
- Samples with hGH levels above the assay range may be prediluted with LIAISON® Endocrinology Diluent (REF 319133).
- Treatment of acromegaly with pegvisomant (recombinant hGH and PEG) increases endogenous serum hGH concentrations 100 to 1000 times and should therefore be monitored based on serum IGF-I levels.
- Integrals may not be exchanged between analyzer types (LIAISON®, LIAISON® XL and LIAISON® XS). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted. Due to traceability issues resulting from the above statement, patient follow-ups may not be concluded between analyzer types. These must be accomplished on one particular analyzer type (either LIAISON®, LIAISON® XL or LIAISON® XS).

15. SPECIFIC PERFORMANCE CHARACTERISTICS

15.1. Analytical specificity

Analytical specificity may be defined as the ability of the assay to accurately detect specific analyte in the presence of potentially interfering factors in the sample matrix (e.g., haemolysis, lipaemia, bilirubinaemia).

Interference. Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by concentrations of bilirubin up to 20 mg/dL, haemoglobin up to 1000 mg/dL or triglycerides up to 5000 mg/dL.

Cross-reactions. The presence of the following potentially cross-reactive molecules did not show any interference in the assay when spiking an hGH-free serum as shown in the following table.
The test was repeated by spiking with the same molecules a specimen containing 7 ng/mL hGH, which is generally considered the threshold concentration to a challenge test, below which a treatment with hGH is suggested in adolescents. The results showed no interference by hFSH, hLH, hPRL, hTSH and β-hCG. Conversely, hCS (hPL) showed a negative bias: hCS, when added in concentrations generally observed in a normal pregnancy (5-15 µg/mL) mimicked a negligible hGH concentration, caused by sequestration of one of the antibodies and consequent hindered formation of the antigen-antibody reticulum.
The test was performed in accordance with the guidelines of Clinical and Laboratory Standards Institute (CLSI, USA), Document No. EP07-A2.

Compound	Spiked amount	% Cross-reactivity
Follicle-stimulating hormone (hFSH)	up to 500 mIU/mL	undetectable
Luteinizing hormone (hLH)	up to 500 mIU/mL	undetectable
Prolactin (hPRL)	up to 1000 ng/mL	undetectable
Thyroid-stimulating hormone (hTSH)	up to 500 µIU/mL	undetectable
Chorionic somatomammotrophin (hCS, hPL)	up to 100 µg/mL	undetectable
Beta-chorionic gonadotrophin (β-hCG)	up to 10 µg/mL	undetectable

15.2. Precision with LIAISON® Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to estimate repeatability and reproducibility of the assay (i.e., within- and between-assay variability). The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

Repeatability. Twenty replicates were performed in the same run to evaluate in-house repeatability.

Repeatability	A	B	C	D	E	Control 1	Control 2
Number of determinations	20	20	20	20	20	20	20
Mean (ng/mL)	0.41	1.19	3.65	10.90	20.53	3.56	16.72
Standard deviation (ng/mL)	0.02	0.03	0.06	0.23	0.42	0.10	0.39
Coefficient of variation (%)	4.4	2.4	1.6	2.1	2.0	2.7	2.4
Min value (ng/mL)	0.39	1.13	3.52	10.55	19.51	3.39	15.78
Max value (ng/mL)	0.47	1.24	3.76	11.31	21.12	3.83	17.37

Reproducibility. Twenty replicates were performed in different days (one or two runs per day) with three different lots of integral to evaluate reproducibility. The tests were performed in two sites, in house (site 1) and in an independent laboratory (site 2) using the same instruments.

Reproducibility - Site 1	A	B	C	D	E	Control 1	Control 2
LOT No. 01							
Number of determinations	20	20	20	20	20	20	20
Mean (ng/mL)	0.35	1.10	3.24	10.14	20.86	3.38	16.57
Standard deviation (ng/mL)	0.02	0.08	0.25	0.71	1.41	0.32	0.98
Coefficient of variation (%)	6.0	7.2	7.7	7.0	6.8	9.4	5.9
Min value (ng/mL)	0.31	0.98	2.82	8.60	18.36	2.96	15.34
Max value (ng/mL)	0.39	1.24	3.69	11.42	23.69	4.14	18.73
LOT No. 02							
Number of determinations	20	20	20	20	20	20	20
Mean (ng/mL)	0.37	1.12	3.29	10.00	19.14	3.34	15.60
Standard deviation (ng/mL)	0.02	0.08	0.24	0.74	1.40	0.27	0.78
Coefficient of variation (%)	5.1	7.6	7.3	7.4	7.3	8.0	5.0
Min value (ng/mL)	0.32	1.00	2.87	7.79	16.63	2.82	14.23
Max value (ng/mL)	0.41	1.30	3.71	11.52	22.17	3.84	17.28
LOT No. 03							
Number of determinations	20	20	20	20	20	20	20
Mean (ng/mL)	0.41	1.24	3.49	10.72	20.68	3.90	17.17
Standard deviation (ng/mL)	0.04	0.13	0.43	0.87	1.60	0.27	1.18
Coefficient of variation (%)	10.6	10.6	12.3	8.1	7.7	6.9	6.9
Min value (ng/mL)	0.32	1.04	2.67	9.64	17.28	3.31	14.46
Max value (ng/mL)	0.48	1.48	4.60	12.76	23.43	4.31	19.73
Inter-lot coefficient of variation (%)	8.3	6.6	3.9	3.7	4.7	8.9	4.8

Reproducibility - Site 2	A	B	C	D	E	Control 1	Control 2
LOT No. 01							
Number of determinations	20	20	20	20	20	20	20
Mean (ng/mL)	0.33	1.03	2.93	8.65	17.35	3.12	15.38
Standard deviation (ng/mL)	0.04	0.14	0.31	0.73	1.99	0.20	0.92
Coefficient of variation (%)	13.5	14.0	10.7	8.5	11.5	6.5	6.0
Min value (ng/mL)	0.23	0.67	2.18	7.31	12.39	2.64	12.61
Max value (ng/mL)	0.38	1.26	3.43	10.02	20.04	3.41	16.77
LOT No. 02							
Number of determinations	20	20	20	20	20	20	20
Mean (ng/mL)	0.32	1.00	2.96	8.98	17.54	3.18	14.94
Standard deviation (ng/mL)	0.04	0.09	0.28	0.92	2.16	0.21	1.44
Coefficient of variation (%)	12.6	8.9	9.5	10.3	12.3	6.5	9.7
Min value (ng/mL)	0.20	0.84	2.13	7.50	12.78	2.69	12.20
Max value (ng/mL)	0.36	1.16	3.30	10.59	20.69	3.50	16.65
LOT No. 03							
Number of determinations	20	20	20	20	20	20	20
Mean (ng/mL)	0.31	0.99	2.96	9.13	17.61	3.45	15.90
Standard deviation (ng/mL)	0.04	0.10	0.32	0.86	1.66	0.26	0.91
Coefficient of variation (%)	11.8	10.3	10.8	9.4	9.4	7.5	5.7
Min value (ng/mL)	0.23	0.79	2.31	7.67	14.86	2.99	14.20
Max value (ng/mL)	0.37	1.16	3.31	10.64	20.38	3.93	17.80
Inter-lot coefficient of variation (%)	2.1	2.0	0.6	2.8	0.8	5.4	3.1

15.3. Precision with LIAISON® XL Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to estimate repeatability and reproducibility of the assay (i.e., within- and between-assay variability). The variability shown in the tables below did not result in sample misclassification.

Repeatability. Twenty replicates were performed in the same run to evaluate repeatability.

Repeatability	1	2	3	4	5	6	7	Control 1	Control 2
Number of determinations	20	20	20	20	20	20	20	20	20
Mean (ng/mL)	0.265	0.730	1.18	4.44	7.84	20.0	41.2	3.48	17.3
Standard deviation (ng/mL)	0.013	0.018	0.023	0.11	0.18	0.47	1.38	0.079	0.33
Coefficient of variation (%)	4.73	2.48	1.93	2.52	2.29	2.37	3.35	2.26	1.93
Min. value (ng/mL)	0.237	0.691	1.14	4.29	7.40	19.3	38.3	3.32	16.8
Max. value (ng/mL)	0.287	0.762	1.23	4.72	8.12	20.9	44.2	3.65	17.9

Reproducibility. Twenty replicates were performed in different days (one or two runs per day) to evaluate reproducibility.

Reproducibility	1	2	3	4	5	6	7	Control 1	Control 2
Number of determinations	20	20	20	20	20	20	20	20	20
Mean (ng/mL)	0.252	0.695	1.11	4.01	5.99	17.3	35.5	3.29	16.2
Standard deviation (ng/mL)	0.016	0.028	0.042	0.16	0.26	0.75	1.70	0.086	0.34
Coefficient of variation (%)	6.25	4.09	3.77	3.97	3.76	4.36	4.79	2.61	2.11
Min. value (ng/mL)	0.223	0.645	1.04	3.67	5.62	16.2	32.1	3.08	15.6
Max. value (ng/mL)	0.281	0.737	1.21	4.20	6.49	18.8	38.1	3.47	17.0

15.4. Precision with LIAISON® XS Analyzer

A five-day precision study was conducted on three LIAISON® XS Analyzers to verify the precision with the LIAISON® hGH Assay. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

A coded panel comprised of seven (7) frozen samples was used for the study.

The LIAISON® Control hGH set was also included in the five-day study.

The coded panel was tested on three LIAISON® XS Analyzers, in six replicates in a single run per day, for 5 operative days.

The mean Index value, standard deviation, and coefficient of variation (%CV) of the results were computed for each of the tested specimens for each of the instruments and across instruments.

Repeatability. Ninety replicates were performed in the same test to evaluate repeatability. 7 serum samples containing different concentration of analyte and kit controls were assayed in 6 replicates per day, over 5 operating days, on 3 units and one reagent lot..

Repeatability	8	9	10	11	12	13	14	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (ng/mL)	51.4	19.2	6.76	2.06	0.825	0.385	0.253	3.88	19.4
Standard deviation	1.020	0.321	0.100	0.033	0.013	0.008	0.008	0.077	0.338
Coefficient of variation (%)	2.0	1.7	1.5	1.6	1.6	2.0	3.2	1.4	0.9
Min. value (ng/mL)	48.6	18.3	6.34	1.90	0.757	0.332	0.216	3.63	18.3
Max. value (ng/mL)	56.7	20.7	7.00	2.21	0.917	0.424	0.302	4.17	21.0

Reproducibility. Ninety replicates were performed in different days (one run per day) to evaluate reproducibility. 7 serum samples containing different concentration of analyte and kit controls were assayed in 6 replicates per day, over 5 operating days, on 3 units and one reagent lot.

Reproducibility	8	9	10	11	12	13	14	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (ng/mL)	51.4	19.2	6.76	2.06	0.825	0.385	0.253	3.88	19.4
Standard deviation	1.950	0.413	0.126	0.045	0.026	0.013	0.014	0.117	0.595
Coefficient of variation (%)	3.8	2.1	1.9	2.2	3.1	3.4	5.5	3.0	3.1
Min. value (ng/mL)	48.6	18.3	6.34	1.90	0.757	0.332	0.216	3.63	18.3
Max. value (ng/mL)	56.7	20.7	7.00	2.21	0.917	0.424	0.302	4.17	21.0

15.5. Linearity by dilution test

Six serum samples with high hGH levels were tested as such and after serially diluting with an hGH-free serum (left columns) or with the calibrator matrix (right columns). Measured versus expected hGH levels were analyzed by linear regression. The correlation coefficients (r) ranged from 0.999 to 1.000.

Dilution	Expected concentration, ng/mL	Measured concentration, ng/mL	% Recovery	Dilution	Expected concentration, ng/mL	Measured concentration, ng/mL	% Recovery
neat	–	15.36	–	neat	–	15.36	–
1:2	7.69	7.51	97.7	1:2	7.68	7.56	98.4
1:4	3.85	3.72	96.7	1:4	3.84	3.73	97.1
1:8	1.93	1.93	100.0	1:8	1.92	1.86	96.9
1:16	0.97	1.00	103.1	1:16	0.96	0.91	94.8
1:32	0.49	0.59	120.2	1:32	0.48	0.49	102.1
mean	–	–	**103.5**	**mean**	–	–	**97.9**
neat	–	32.47	–	neat	–	32.47	–
1:2	16.24	15.86	97.7	1:2	16.24	16.24	100.0
1:4	8.13	7.31	90.0	1:4	8.12	7.56	93.1
1:8	4.07	3.66	90.0	1:8	4.06	3.69	90.9
1:16	2.04	1.85	90.7	1:16	2.03	1.75	86.2
1:32	1.03	0.96	93.6	1:32	1.01	0.99	97.6
1:64	0.51	0.49	96.6	1:64	0.51	0.48	94.6
mean	–	–	**93.1**	**mean**	–	–	**93.7**
neat	–	50.49	–	neat	–	50.49	–
1:2	25.26	24.62	97.5	1:2	25.25	26.14	103.5
1:4	12.65	12.76	100.9	1:4	12.62	12.47	98.8
1:8	6.34	6.26	98.8	1:8	6.31	6.36	100.8
1:16	3.18	3.24	101.8	1:16	3.16	3.18	100.8
1:32	1.61	1.66	103.3	1:32	1.58	1.68	106.5
1:64	0.82	0.81	99.0	1:64	0.79	0.80	101.4
mean	–	–	**100.2**	**mean**	–	–	**102.0**

Two additional serum samples with hGH levels above the assay range were tested as such and after serially diluting with LIAISON® Endocrinology Diluent. Measured versus expected hGH levels were analyzed by linear regression. The correlation coefficients (r) were 0.999.

Dilution	Expected concentration, ng/mL	Measured concentration, ng/mL	% Recovery	Dilution	Expected concentration, ng/mL	Measured concentration, ng/mL	% Recovery
neat	–	> 80.00	–	neat	–	> 80.00	–
1:2	–	39.02	–	1:2	–	63.16	–
1:4	19.51	20.18	103.4	1:4	31.58	31.53	99.8
1:8	9.76	10.23	104.9	1:8	15.79	16.89	107.0
1:16	4.88	5.36	109.9	1:16	7.90	7.57	95.9
1:32	2.44	2.77	113.6	1:32	3.95	3.65	92.5
mean	–	–	**107.9**	**mean**	–	–	**98.8**

15.6. Trueness by recovery test

Four sets formed of a high- and a low- to normal-hGH sample (samples A and B in set 1 - samples C and D in set 2 - samples E and F in set 3 - samples G and H in set 4) were mixed in 1:5, 1:2, 1:1, 2:1 and 5:1 ratios and assayed. Percent recoveries were determined from results of undiluted samples. Measured versus expected hGH concentrations were analyzed by linear regression. The correlation coefficients (r) ranged from 0.999 to 1.000.

Set	Expected concentration, ng/mL	Measured concentration, ng/mL	% Recovery	Set	Expected concentration, ng/mL	Measured concentration, ng/mL	% Recovery
A neat	–	6.96	–	C neat	–	0.18	–
5:1	14.29	14.94	104.5	5:1	1.71	1.96	114.5
2:1	21.62	21.47	99.3	2:1	3.24	3.24	99.9
1:1	28.96	29.12	100.6	1:1	4.78	4.71	98.6
1:2	36.29	36.70	101.1	1:2	6.31	6.72	106.6
1:5	43.62	44.73	102.5	1:5	7.84	8.07	103.0
B neat	–	50.95	–	D neat	–	9.37	–
E neat	–	1.72	–	G neat	–	2.96	–
5:1	3.97	4.05	101.9	5:1	6.50	5.93	91.3
2:1	6.23	6.32	101.5	2:1	10.03	9.48	94.5
1:1	8.48	8.05	94.9	1:1	13.57	13.40	98.7
1:2	10.73	10.45	97.4	1:2	17.11	16.74	97.9
1:5	12.99	13.21	101.7	1:5	20.64	21.25	102.9
F neat	–	15.24	–	H neat	–	24.18	–

15.7. High-dose hook effect

The high-dose hook effect (HDH) was determined by addition of recombinant hGH to an hGH-free serum up to a maximum of 7,000 ng/mL.
Whenever samples containing extremely high analyte concentrations are tested, the high-dose hook effect can mimic concentrations lower than real. Analysis of high-dose hook effect was evaluated by testing an hGH-free serum spiked with high concentrations of hGH. All samples resulted in calculated concentration values above the assay range, indicating no sample misclassification.

15.8. Analytical and functional sensitivity

Analytical sensitivity (detection limit) is defined as the minimum detectable dose that can be distinguished from zero.

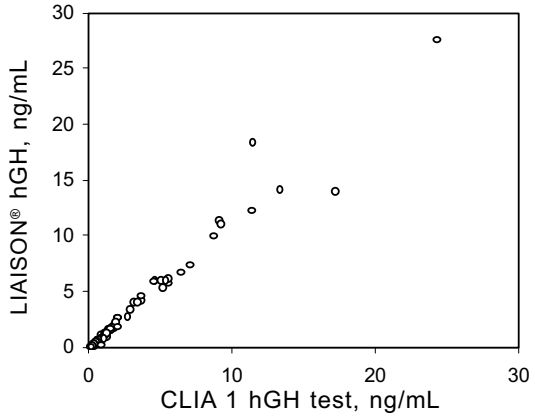
Analytical sensitivity, calculated in accordance with the guidelines of Clinical and Laboratory Standards Institute (CLSI, USA), Document No. EP17-A, ranges from 0.095 ng/mL to 0.100 ng/mL (as assessed by several assay runs, kit lots and instruments).

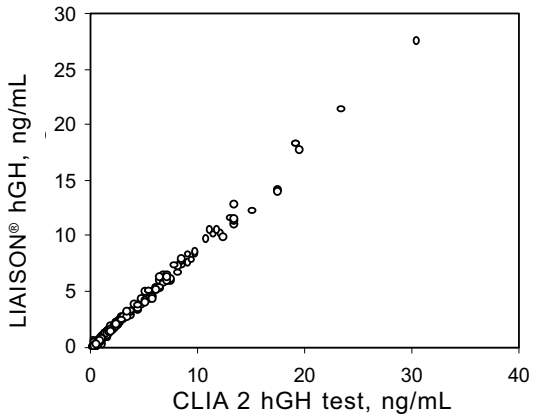
Analytical sensitivity, defined as the minimum detectable dose that can be distinguished from zero by two standard deviations (that is, two standard deviations above zero), ranges from 0.009 ng/mL to 0.052 ng/mL (as assessed by several assay runs, kit lots and instruments).

Functional sensitivity, defined as the concentration at which the between-assay coefficient of variation (CV) exceeds 20%, is less than 0.05 ng/mL (as assessed by several assay runs, kit lots and instruments).

15.9. Method comparison

The LIAISON® hGH results were compared with those of two reference CLIA methods. The following correlations were obtained by linear regression analysis:
LIAISON® hGH = 1.118 x reference CLIA 1 method + 0.074. Correlation coefficient r = 0.981 (n = 85).
LIAISON® hGH = 0.898 x reference CLIA 2 method + 0.004. Correlation coefficient r = 0.997 (n = 252).





REFERENCES

R.L. ABRAMS et al.
Hypothalamic regulation of growth hormone secretion.
J. Clin. Invest., **43** : 1242 (1964).

G. BANFI, M. MARINELLI, M. PONTILLO, P. BONINI
Standardisation with synthetic 22-kDa monomer human growth hormone reduces discrepancies between two monoclonal immunoradiometric assay kits.
Clin. Chem., **38** (10) : 2107-2110 (1992).

R. BOUILLON, P. DE MOOR
Heterogeneity of human growth hormone in serum.
Ann. Endocr., **35** : 606 (1974).

A.C. CELNIKER, A.B. CHEN, R.M. WERT Jr., B.M. SHERMAN
Variability in the quantitation of circulating growth hormone using commercial immunoassays.
J. Clin. Endocrinol. Metab., **68** : 469-475 (1989).

E. GHIGO et al.
Growth hormone-releasing activity of hexarelin, a new synthetic hexapeptide, after intravenous, subcutaneous, intranasal, and oral administration in man.
J. Clin. Endocrinol. Metab., **78** : 693-698 (1994).

W.M. HUNTER
Growth hormone.
In: Hormone Assays and Their Clinical Application, J.A. Loraine, E.T. Bell eds., Churchill Livingstone, Edinburgh, p. 221 (1976).

U.J. LEWIS et al.
Human growth hormone: a complex of proteins.
Rec. Progr. Horm. Res., **36** : 477 (1980).

C.H. LI
Human growth hormone: perspectives on its chemistry and physiology.
In: Advances in Human Growth Hormone Research, S. Raiti ed., U.S. Department of Health, Education and Welfare Publ., DHEW publ. no. (NIH) 74-612, p. 321 (1974).

G.M. MOLINATTI et al.
Radioimmunoassay of human growth hormone.
J. Nucl. Biol. Med., **13** : 26 (1968).

G.T. PEAKE, J. MORRIS, M.T. BUCKMAN
Growth hormone.
In: Methods of Hormone Radioimmunoassay, B.M. Jaffe, H.R. Behrman eds., Academic Press, New York, p. 223 (1979).

E. SCHAFF-BASS et al.
Advances in diagnosis and treatment of short stature with special reference to the role of growth hormone.
J. Pediatr., **104** : 801 (1984).

M.O. THORNER, M.L. VANCE, E.R LAWS Jr., E. HORVATH, K. KOVACS
The anterior pituitary.
In: Textbook of Endocrinology, R.H. Williams ed., 9th edition, W.B. Saunders, Philadelphia, p. 249 (1998).

B.T. TRUDD
Growth, growth hormone and the somatomedins: a historical perspective and current concepts.
Ann. Clin. Biochem., **28** : 542-555 (1991).

200/007-907, 07 - 2024-04