Hướng dẫn sử dụng tiếng Anh

Tài liệu được xác nhận bằng chữ ký số

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Người đại diện hợp pháp của cơ sở

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Changes: §4, §6, §7;
Deletions: -

LIAISON® Insulin (REF 310360)

1. INTENDED USE

The LIAISON® Insulin assay uses chemiluminescence immunoassay (CLIA) technology for the *in vitro* quantitative determination of insulin in human serum or EDTA-plasma specimens.
The test has to be performed on the LIAISON® Analyzer family.

2. SUMMARY AND EXPLANATION OF THE TEST

Insulin is a polypeptide hormone of molecular weight 6 kDa, composed of two peptide chains, A (21 amino acids) and B (30 amino acids), jointed by two cross-linked disulphide bonds and synthesized by the beta cells of the islets of Langerhans of the pancreas. Insulin is cleaved from a single-chain precursor, proinsulin (9-kDa molecular weight), where the A and B chains are jointed together by a connecting peptide (C-peptide). Specific proteases cleave proinsulin to insulin and C-peptide, which are stored in granules in the cells of the islets of Langerhans of the pancreas and are then secreted simultaneously into the bloodstream. Insulin is primarily secreted as direct response to stimulation by exogenous glucose.

Insulin influences most of the metabolic functions of the body. Its best known action is to lower the blood glucose concentration by increasing the rate at which glucose is converted to glycogen in the liver and muscles and to fat in adipose tissue, by stimulating the rate of glucose metabolism and by depressing gluconeogenesis.
Insulin stimulates the synthesis of proteins, DNA and RNA in cells generally, and promotes the uptake of amino acids and their incorporation into muscle protein. It increases the uptake of glucose in adipose tissue and its conversion into fat and inhibits lipolysis. Insulin primary action is on the cell membrane, where it probably facilitates the transport of glucose and amino acids into the cells. At the same time it may activate intracellular enzymes such as glycogen synthetase, concerned with glycogen synthesis.

Insulin immunoassay is of help in the diagnosis of diabetes mellitus and other carbohydrate and lipid metabolism disorders. Increased insulin levels are observed in obese subjects, in women treated with oral contraceptives, and in patients affected by Cushing's syndrome, acromegaly, hyperthyroidism. Decreased insulin levels are found mainly in diabetes mellitus. Circulating insulin concentrations are generally investigated by glucose tolerance test or fasting blood glucose levels. A single random blood determination, however, may not provide sufficient information due to the wide fluctuations in time response of insulin and blood glucose levels in different individuals and clinical conditions.

3. PRINCIPLE OF THE PROCEDURE

The method for the quantitative determination of insulin is a sandwich chemiluminescence immunoassay.
A specific mouse monoclonal antibody to insulin is coated on the magnetic particles (solid phase); another monoclonal antibody (specific for a different epitope of the insulin molecule) is linked to an isoluminol derivative (isoluminol-antibody conjugate).
During the incubation, insulin present in calibrators, samples or controls binds to the solid phase monoclonal antibody, and subsequently the antibody conjugate reacts with insulin already bound to the solid phase. A sandwich is formed only in the presence of insulin 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 insulin concentration present in calibrators, samples or controls.

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-insulin monoclonal antibody (mouse), bovine serum albumin, PBS buffer, < 0.1% sodium azide.
Conjugate (28 mL)	CONJ	Anti-insulin monoclonal antibody (mouse), labelled with isoluminol derivative, non-specific mouse IgG, bovine serum albumin, PBS buffer, detergents, 0.2% ProClin™ 300, preservatives.

Included in the kit:

Calibrator A (1.5 mL)	CAL A	Semisynthetic human insulin (prepared enzymatically from pig insulin), human insulin-free serum, 0.2% ProClin™ 300, <1% gentamycin sulfate salt. (lyophilized reagent)
Calibrator B (1.5 mL)	CAL B	semisynthetic human insulin (prepared enzymatically from pig insulin), human insulin-free serum, an inert blue dye, 0.2% ProClin™ 300, <1% gentamycin sulfate salt. (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). LIAISON® XL Starter Kit (REF 319200). – 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). LIAISON® Light Check 12 (REF 319150). LIAISON® Wash/System Liquid (REF 319100). LIAISON® Waste Bags (REF 450003). LIAISON® Cleaning Kit (REF 310990).

Additionally required materials

LIAISON® Insulin controls, levels 1 and 2 (REF 310361).
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 Analyzer

– LIAISON® XL Analyzer is 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® Insulin 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 transferred to a 12 x 75mm polystyrene tube. Affix the proper bar-coded label to the calibrator tube and load on to the instrument. Each calibrator solution allows at least seven 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 Analyzer 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® Insulin 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 three days when properly stored at 2-8°C either in their sealed vials or in stoppered transfer tubes; for longer storage, calibrators should be stored deep-frozen (–20°C or below). After reconstitution the calibrators must be stored in an upright position to prevent adherence of the solution to the vial or tube cap. When stored frozen, mix thawed calibrators well before testing. The results show no significant differences when calibrators go through three freeze-thaw cycles.

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

Either human serum or EDTA-plasma may be used in the assay. Use of plasma samples other than EDTA-plasma 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). Ten samples underwent four 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 samples immediately after loading on to the instrument.
The minimum volume required for a single determination is 210 µL specimen (60 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 seven 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 four weeks before.
– Each time a new lot of integral is used.
– The Analyzer has been serviced.
– 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).

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 Analyzer. 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® Insulin 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 reconstituted 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 insulin concentrations for the unknown samples. For details, refer to the relevant Analyzer Operator's Manual.
Calibrators and controls may give different RLU or dose results on LIAISON® and LIAISON® XL, but patient results are equivalent.

Assay range: The Analyzer directly calculates insulin concentration up to 500 µIU/mL.

Reference standard: The assay is referenced to the World Health Organization First International Reference Preparation, code NIBSC 66/304.

Expected values: Each laboratory should establish its own range of expected values for the population taken into consideration.

Clinical data: A study was performed in a clinical centre in Germany in 101 individuals, that yielded the results showed in the following table.
Serum samples were collected from selected fasting adult subjects. Individuals were assigned to subgroups based on the results of a standardized oral glucose tolerance test (oGTT), performed in accordance with the criteria for diagnosis of diabetes mellitus established by the American Diabetes Association (ADA).
The group formed of 68 apparently healthy subjects with normal glucose tolerance test showed median basal insulin levels of 7.69 µIU/mL (5th-95th percentile: 3.21-16.32 µIU/mL).
These results refer to the groups of samples investigated and are not guaranteed specifications, but are indicative only.

Subject classification	Number of subjects (F / M)	Age range (years)	Insulin median value (µIU/mL)		
			Glucose administration	30 min post-administration	120 min post-administration
Apparently healthy subjects with normal glucose tolerance test (NGT)	68 (36/32)	18.0-79.2	7.69	50.69	25.49
Subjects with impaired glucose tolerance test (IGT)	18 (10/8)	40.0-82.0	13.56	77.61	83.95
Subjects with diabetes mellitus (DM)	15 (11/4)	46.6-81.0	10.57	41.32	60.33

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.
- Test results are reported quantitatively. However, diagnosis of a disease should be established based on the patient's anamnesis, in conjunction with clinical findings and in association with medical judgement. Any therapeutical decision must also be taken on a case-by-case basis.
- The LIAISON® Insulin 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.
- The presence of circulating insulin autoantibodies may interfere in the assay.
- No interference due to drug administration has been investigated.
- Samples with insulin levels above the assay range may be prediluted with LIAISON® Endocrinology Diluent (REF 319133).
- Two comparison studies were carried out with two kit lots in 40 paired sample sets of serum and EDTA-plasma. The following correlations were obtained by linear regression analysis.
  EDTA-plasma result = 1.116 x serum result –1.522. Correlation coefficient r = 0.995.
  EDTA-plasma result = 1.241 x serum result –3.987. Correlation coefficient r = 0.991.
- Integrals may not be exchanged between analyzer types (LIAISON® and LIAISON® XL). 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® or LIAISON® XL).

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 3000 mg/dL.

Cross-reactions. The presence of the following potentially cross-reactive molecules causes interference in the assay as shown in the table below. Such interference, however, has limited clinical relevance. 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
Human C-peptide	200 ng/mL	–1.4
Human proinsulin	200 ng/mL	–0.9
Human glucagon	200 ng/mL	–0.8
Insulin-like growth factor I (IGF-I)	200 ng/mL	2.9
Bovine insulin	0.5 ng/mL	74.7
Porcine insulin	0.5 ng/mL	191.3

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 (µIU/mL)	4.7	8.7	22.7	45.3	120.0	11.6	108.3
Standard deviation (µIU/mL)	0.2	0.3	0.6	0.8	4.7	0.4	2.2
Coefficient of variation (%)	4.3	3.3	2.6	1.8	3.9	3.0	2.0
Min value (µIU/mL)	4.4	8.2	21.8	43.6	114.2	10.8	102.8
Max value (µIU/mL)	5.1	9.3	24.2	46.7	133.3	12.1	112.3

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	F	G	H	I	J	Control 1	Control 2
LOT No. 01							
Number of determinations	20	20	20	20	20	20	20
Mean (µIU/mL)	8.2	20.0	42.4	88.7	139.4	10.7	107.3
Standard deviation (µIU/mL)	0.5	0.9	1.9	4.4	4.7	0.9	5.4
Coefficient of variation (%)	5.5	4.4	4.5	5.0	3.3	8.1	5.0
Min value (µIU/mL)	7.3	18.6	39.8	83.1	134.4	9.5	97.7
Max value (µIU/mL)	9.1	21.6	47.1	101.1	148.1	12.5	116.7
LOT No. 02							
Number of determinations	20	20	20	20	20	20	20
Mean (µIU/mL)	8.5	20.7	43.4	88.5	137.6	11.0	107.5
Standard deviation (µIU/mL)	0.6	1.3	3.3	4.6	6.5	0.7	5.1
Coefficient of variation (%)	6.9	6.0	7.7	5.2	4.7	6.6	4.7
Min value (µIU/mL)	7.3	18.1	36.8	78.0	127.3	9.6	97.7
Max value (µIU/mL)	9.7	22.6	47.8	94.2	146.5	12.2	116.5
LOT No. 03							
Number of determinations	20	20	20	20	20	20	20
Mean (µIU/mL)	7.9	19.3	40.9	86.1	137.5	10.1	105.6
Standard deviation (µIU/mL)	0.4	1.1	3.3	5.3	7.4	0.6	7.2
Coefficient of variation (%)	5.4	5.7	8.2	6.2	5.4	5.7	6.9
Min value (µIU/mL)	7.2	17.0	34.9	76.1	120.9	9.1	94.3
Max value (µIU/mL)	8.5	20.9	44.7	92.5	149.0	11.0	117.4
Inter-lot coefficient of variation (%)	3.7	3.5	3.0	1.6	0.8	4.3	1.0

Reproducibility - Site 2	K	L	M	N	O	Control 1	Control 2
LOT No. 01							
Number of determinations	20	20	20	20	20	20	20
Mean (µIU/mL)	4.5	9.1	33.1	98.8	153.4	8.9	115.9
Standard deviation (µIU/mL)	0.3	0.9	2.1	7.5	6.6	0.3	3.3
Coefficient of variation (%)	5.5	9.9	6.2	7.5	4.3	3.9	2.8
Min value (µIU/mL)	3.9	5.9	30.0	84.1	143.6	8.1	109.9
Max value (µIU/mL)	5.0	10.1	37.9	112.5	171.0	9.4	120.9
LOT No. 02							
Number of determinations	20	20	20	20	20	20	20
Mean (µIU/mL)	3.7	7.4	29.2	93.8	142.4	8.3	103.9
Standard deviation (µIU/mL)	0.3	0.5	1.6	8.6	9.3	0.4	6.1
Coefficient of variation (%)	8.1	6.7	5.4	9.2	6.5	4.9	5.9
Min value (µIU/mL)	3.0	6.3	25.4	68.0	111.3	7.4	90.8
Max value (µIU/mL)	4.2	8.0	31.6	104.0	153.5	9.2	117.0
LOT No. 03							
Number of determinations	20	20	20	20	20	20	20
Mean (µIU/mL)	3.8	8.2	30.8	95.7	147.9	7.8	105.1
Standard deviation (µIU/mL)	0.2	0.3	1.9	8.2	6.3	0.3	3.6
Coefficient of variation (%)	4.7	3.7	6.1	8.5	4.3	4.4	3.5
Min value (µIU/mL)	3.5	7.7	25.4	74.3	128.9	7.1	98.6
Max value (µIU/mL)	4.1	8.8	33.4	109.9	155.0	8.4	110.3
Inter-lot coefficient of variation (%)	11.6	10.4	6.3	2.6	3.7	6.8	6.1

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 (µIU/mL)	5.763	7.730	12.03	27.51	69.23	88.03	131.1	10.70	113.2
Standard deviation (µIU/mL)	0.29	0.24	0.32	0.77	1.50	2.57	2.45	0.39	6.89
Coefficient of variation (%)	4.95	3.09	2.64	2.79	2.16	2.92	1.87	3.62	6.09
Min. value (µIU/mL)	5.470	7.328	11.34	25.74	65.07	82.82	124.9	9.826	98.33
Max. value (µIU/mL)	6.384	8.058	12.57	28.64	71.28	92.09	135.0	11.13	120.8

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 (µIU/mL)	3.914	7.765	11.81	27.08	69.93	88.87	131.8	10.49	112.4
Standard deviation (µIU/mL)	0.19	0.40	0.51	1.03	2.11	3.60	3.95	0.61	5.24
Coefficient of variation (%)	4.73	5.17	4.35	3.81	3.02	4.05	3.00	5.84	4.66
Min. value (µIU/mL)	3.333	7.048	10.76	24.44	66.37	80.64	126.1	9.342	99.72
Max. value (µIU/mL)	4.298	8.402	12.97	28.89	73.18	93.62	141.0	11.43	120.6

15.4. Linearity by dilution test

Two serum samples with high insulin levels were tested as such and after serially diluting with an insulin-free serum. Measured versus expected insulin levels were analyzed by linear regression. The correlation coefficients (r) were 0.999.

Dilution	Expected concentration, µIU/mL	Measured concentration, µIU/mL	% Recovery	Dilution	Expected concentration, µIU/mL	Measured concentration, µIU/mL	% Recovery
neat	–	153.9	–	neat	–	101.9	–
1:2	77.0	73.8	96.0	1:2	50.9	47.9	94.1
1:4	38.5	39.6	102.9	1:4	25.5	25.7	100.9
1:8	19.2	21.4	111.4	1:8	12.7	13.1	102.7
1:16	9.6	11.8	122.5	1:16	6.4	6.9	107.6
1:32	4.8	5.5	115.0	1:32	3.2	3.2	100.2

Two additional serum samples with insulin levels above the assay range were tested as such and after serially diluting with LIAISON® Endocrinology Diluent. Measured versus expected insulin levels were analyzed by linear regression. The correlation coefficients (r) were 1.000.

Dilution	Expected concentration, µIU/mL	Measured concentration, µIU/mL	% Recovery	Dilution	Expected concentration, µIU/mL	Measured concentration, µIU/mL	% Recovery
neat	–	> 500.0	–	neat	–	> 500.0	–
1:2	–	346.6	–	1:2	–	> 500.0	–
1:4	173.3	170.7	98.5	1:4	–	344.6	–
1:8	86.7	88.1	101.7	1:8	172.3	179.7	104.3
1:16	43.3	45.8	105.7	1:16	86.2	95.3	110.6
1:32	21.7	23.0	106.2	1:32	43.1	49.0	113.8

15.5. Trueness by recovery test

Two sets formed of a high- and a low- to normal-insulin sample (samples X and Y in set 1 - samples W and Z in set 2) 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 insulin concentrations were analyzed by linear regression. The correlation coefficients (r) ranged from 0.993 to 0.999.

Set 1	Expected concentration, µIU/mL	Measured concentration, µIU/mL	% Recovery	Set 2	Expected concentration, µIU/mL	Measured concentration, µIU/mL	% Recovery
X neat	–	4.1	–	W neat	–	20.3	–
5:1	26.6	25.1	94.2	5:1	30.1	29.8	98.8
2:1	49.2	46.2	94.0	2:1	39.9	37.9	94.8
1:1	71.7	72.8	101.4	1:1	49.8	49.0	98.4
1:2	94.3	94.8	100.5	1:2	59.6	62.8	105.4
1:5	116.8	121.5	103.9	1:5	69.4	68.5	98.7
Y neat	–	139.4	–	Z neat	–	79.3	–

15.6. High-dose hook effect

The high-dose hook effect (HDH) was determined by addition of recombinant insulin to an insulin-free serum up to a maximum of 200,000 µIU/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 insulin-free serum spiked with high concentrations of insulin. All samples resulted in calculated concentration values above the assay range, indicating no sample misclassification.

15.7. Analytical and functional sensitivity

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

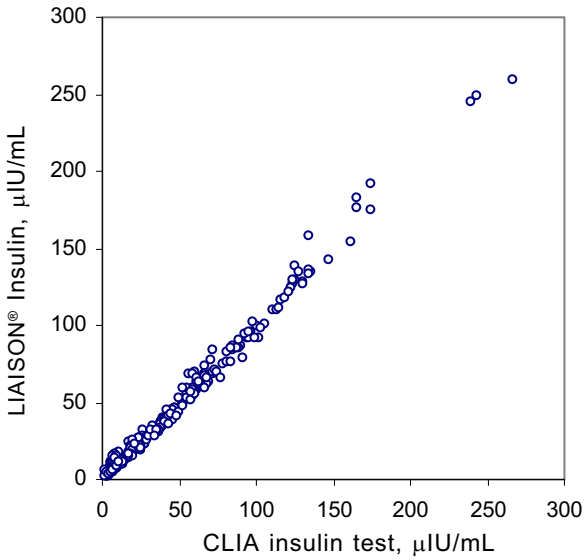
Analytical sensitivity, calculated in accordance with the guidelines of Clinical and Laboratory Standards Institute (CLSI, USA), Document No. EP17-A, ranges from 0.23 µIU/mL to 0.61 µIU/mL (as assessed by several assay runs, kit lots and instruments).

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 0.17 µIU/mL to 0.50 µIU/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%, ranges from 0.51 µIU/mL to 0.87 µIU/mL (as assessed by several assay runs, kit lots and instruments).

15.8. Method comparison

The LIAISON® Insulin results were compared with those of a reference CLIA method in serum samples. The following correlation was obtained by linear regression analysis:
LIAISON® Insulin = 1.012 x reference CLIA method –0.46. Correlation coefficient r = 0.996 (n = 331).



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