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CE

LIAISON® Direct Renin (310470)

1. INTENDED USE

The LIAISON® Direct Renin assay uses chemiluminescent immunoassay (CLIA) technology for the *in vitro* quantitative determination of renin in human EDTA-plasma specimens. Renin measurement is of help in the diagnosis and treatment of a number of hypertension types in humans.
The test has to be performed on the LIAISON® Analyzer.

2. SUMMARY AND EXPLANATION OF THE TEST

The proteolytic enzyme renin (molecular weight: about 42 kDa) is mainly synthesized by the juxtaglomerular cells of the kidneys as prorenin and is stored in granules as prorenin or renin. It is released in response to physiological stimuli like decreased blood volume and blood pressure, and sodium depletion. Renin inactive precursor is prorenin, which is converted into renin by two steps. Firstly, prorenin undergoes a reversible conformational change (which gives rise to activated prorenin); secondly, 46 amino acids of prorenin are proteolytically cleaved to produce active renin, a glycoprotein formed of 340 amino acid residues. Part of prorenin escapes proteolytic cleavage to renin and is released into the circulation. Prorenin can be activated by different ways, like cryoactivation, acidification or partial proteolysis. Prorenin secretion does not appear to be tightly regulated, whereas renin secretion is strictly controlled. Blood concentration of prorenin is approximately ten-fold greater than that of renin. Renin is termed a double-domain enzyme, because the N- and C-terminal ends are quite similar. Each domain contains a single aspartic acid residue, critical for catalytic activity.

Renin catalyzes the formation of angiotensin I (a decapeptide) by proteolytic cleavage of renin substrate, called angiotensinogen, a glycoprotein synthesized in the liver. Angiotensin-converting enzyme (ACE), in turn, converts angiotensin I to angiotensin II, an octapeptide, that promotes aldosterone release and inhibits renin secretion by a negative feedback mechanism. The renin-angiotensin-aldosterone system (RAAS) plays a paramount role in water homeostasis and electrolyte balance, and in the regulation of arterial pressure. Measurement of plasma renin and aldosterone is therefore considered a marker of the renin-angiotensin-aldosterone system activity. Measurement of total renin (prorenin plus active renin) or of prorenin are of lesser clinical interest. Theoretically, angiotensin II may work as a better marker, but angiotensin II has a very short half-life, and it is difficult to distinguish from angiotensin I.

Angiotensinogen, renin substrate, is the limiting factor in angiotensin II production. In fact, its availability contributes to a certain degree to the stimulation or inhibition of the renin-angiotensin-aldosterone system. Angiotensin is not stored in liver cells in which it is synthesized, in contrast to renin, which is stored in kidneys where it is produced. Increased levels of thyroid hormones (hyperthyroidism), estrogens (oral contraceptives, pregnancy) as well as glucocorticoids (Cushing's syndrome, corticotherapy) all lead to increased renin substrate levels.

Angiotensin II is involved in control of glomerular filtration and renal blood flow. Renin is secreted by kidneys in response to reduction in renal artery perfusion (intrarenal baroceptor), reduction in distal tubular resorption of sodium ions (sodium leakage), hypokalaemia or stimulation of β-adrenergic receptors. In addition, renin secretion is reduced (by negative feedback) in the presence of high plasma concentrations of angiotensin II.

Increased renin levels are found in	Lowered renin levels are found in
Secondary aldosteronism (severe hypertension of renal origin). Direct renin measurement helps in differentiation of primary from secondary hyperaldosteronism in conjunction with aldosterone assay.	Primary aldosteronism. Direct renin measurement helps in differentiation of primary from secondary hyperaldosteronism in conjunction with aldosterone assay.
Addison's disease.	Salt-retaining steroid therapy.
Low-sodium diet, administration of diuretics, haemorrhage.	Vasopressin (ADH) therapy.
Chronic renal failure.	Congenital adrenal hyperplasia with 17-hydroxylase deficiency.
Salt-losing status because of gastrointestinal disease.	
Renin-producing kidney tumours.	
Essential hypertension.	
Hypokalaemia.	
Bartter's syndrome (high renin levels without hypertension).	
Renal artery stenosis.	

As a general rule, people with systolic blood pressure consistently above 160 mmHg and/or diastolic blood pressure over 95-100 mmHg (hypertension) need anti-hypertensive treatment. The yearly death rate related to hypertension is estimated as five million worldwide.

Hypertension is of two main types:
Essential or primary hypertension (90-95% overall), where the cause is unknown in origin.
Secondary hypertension (5-10%), e.g. due to an underlying cause, which may have the following origins:
– Hypertension related to kidney failure (2-3% of vascular origin; 2-3% of parenchymal origin).
– Hypertension related to hormonal disorders.
– Hypertension related to endocrine tumours (very rare).
– Iatrogenic hypertension (e.g., due to oral contraceptives, 1%).

Renin should be measured in accordance with medical judgement, whenever:
– Diastolic blood pressure exceeds 90 mmHg (guidelines of the European Society of Hypertension and European Society of Cardiology).
– Systolic blood pressure exceeds 140 mmHg (guidelines of the European Society of Hypertension and European Society of Cardiology).
– Hypokalaemia is present (to establish differential diagnosis of secondary hyperaldosteronism or primary hypermineralocorticism).
– Response to current anti-hypertensive treatment is insufficient.
– Functional character of a renal artery stenosis is investigated (by renin measurement in the renal veins during acute inhibition of angiotensin-converting enzyme).
– Cancer is linked to increased blood pressure (to establish differential diagnosis of ectopic production of renin).

3. PRINCIPLE OF THE PROCEDURE

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

To prevent renin overestimation, sample handling conditions which may activate prorenin must be avoided (see paragraph Specimen Collection and Preparation).

4. MATERIALS PROVIDED

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

Reagent Integral for 100 determinations	
2.3 mL	Magnetic particles (suspension) coated with anti-renin and prorenin monoclonal antibody (mouse), bovine serum albumin, PBS buffer, < 0.1% sodium azide.
13 mL	Conjugate solution: anti-renin monoclonal antibody (mouse), labelled with isoluminol derivative, non-specific mouse IgG, bovine serum albumin, PBS buffer, preservatives.

Included in the kit:

5 x 2.0 mL	Calibrator A (lyophilized): recombinant human renin (active 340-amino acid protease produced in human embryonic kidney cells transfected with a human renin expression construct), phosphate buffer, bovine serum albumin, an inert yellow dye, preservatives.
5 x 2.0 mL	Calibrator B (lyophilized): recombinant human renin (active 340-amino acid protease produced in human embryonic kidney cells transfected with a human renin expression construct), phosphate buffer, bovine serum albumin, an inert blue dye, preservatives.
5 x 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
LIAISON® Module (code 319130).
LIAISON® Starter Kit (code 319102).
LIAISON® Light Check (code 319101).
LIAISON® Wash/System Liquid (code 319100).
LIAISON® Waste Bags (code 450003).

Additionally required materials
LIAISON® Direct Renin controls, levels 1 and 2 (code 310471).
LIAISON® Cleaning Kit (code 310990).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

6. SAFETY PRECAUTIONS

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

Do not pipette solutions by mouth.

Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses and disposable gloves. Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol. Any reagent spills should be washed with a 5% sodium hypochlorite solution and disposed of as though potentially infectious.

All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each Country.

7. REAGENT PREPARATION

REAGENT INTEGRAL

Before removing the seals from the containers, gently and carefully shake the reagent integral horizontally. Avoid formation of foam. Remove the seal from each container and turn the thumb wheel at the bottom of the magnetic particle container to and fro until the suspension turns brown. This procedure initiates resuspension of magnetic particles. Carefully wipe the surface of each septum to remove residual liquid. Then, 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.

CALIBRATORS

LIAISON® Direct Renin calibrators are supplied lyophilized.

– Reconstitute the vial contents with 2.0 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 three 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 LIAISON® Operator's Manual.
Renin concentrations in the calibrators are printed on the vial labels, coded in the bar-coded labels provided separately and in the reagent integral bar codes.

CONTROLS

Refer to the LIAISON® Direct Renin 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 six 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 LIAISON® Analyzer for a reagent integral already opened.
– Use storage rack provided with the LIAISON® 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 two 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.

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

9. SPECIMEN COLLECTION AND PREPARATION

When prorenin, the inactive precursor of renin, is cryoactivated to renin during sample handling, falsely elevated results are obtained. Cryoactivation occurs when patient samples are chilled to temperatures of 4°C or below for extended periods of time, and when samples are chilled but still liquid (i.e., not frozen). Cryoactivation of prorenin to renin occurs more rapidly in serum. Prorenin blood concentration is approximately ten-fold greater than that of renin.

The only sample material validated is human EDTA-plasma. Use of serum, heparinized plasma, and citrated plasma samples provides lower renin values, and is therefore not recommended.
Careful standardization of the patient preparation and sampling conditions is strongly recommended. Collect blood at room temperature by venipuncture, in siliconized glass tubes, vacutainers (violet cap) or equivalent, containing EDTA as anticoagulant. The presence of haemolysis may indicate mistreating during sample collection or handling.
Fasting specimens are recommended but not required. Record the time of day and the patient's posture during blood collection (supine, upright or seated). Do not pre-chill EDTA blood collection tubes nor store tubes on ice, but process blood at room temperature. Centrifuge tubes in a non-refrigerated centrifuge, separate EDTA-plasma from cells immediately after centrifugation, then aliquot and deep-freeze at –20°C or below instantly.

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.
Avoid repeated freeze-thaw cycles.
It is recommended to test plasma samples immediately after loading on to the instrument.
The minimum volume required for a single determination is 350 µL specimen (200 µL specimen + 150 µL dead volume).

10. CALIBRATION

Assay of calibrators contained in the reagent integral box allows the Analyzer to recalibrate the stored master curve, as indicated via the bar codes on the reagent integral label.

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.

The Analyzer should be calibrated in triplicate whenever 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.
– The Analyzer has been serviced.
– Control values lie outside the expected ranges.

11. ASSAY PROCEDURE

Strict adherence to the Analyzer Operator's Manual ensures proper assay performance. Each test parameter is identified via the bar codes printed on both the reagent integral label and the bar-coded calibrator labels. In case of malfunction of the bar code reader, the data can be entered manually. For details, refer to the Analyzer Operator's Manual.

The Analyzer operations are as follows:

200 µL	Calibrators, controls or specimens.
+ 20 µL	Coated magnetic particles.
+ 100 µL	Conjugate.
31.5 minutes	Incubation followed by a wash cycle.
3 seconds	Measurement.

12. QUALITY CONTROL

Quality control must be performed by running LIAISON® Direct Renin 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 six weeks, or in agreement with guidelines or requirements of local regulations or accredited organizations.

Warning: LIAISON® controls should be run in singlicate to monitor the assay performance. 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 a freshly 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 renin concentrations for the unknown samples. For details, refer to the Analyzer Operator's Manual.

Measuring range: The Analyzer directly calculates renin concentration up to 500 µIU/mL.

Reference standard: The assay is referenced to the World Health Organization International Reference Preparation, NIBSC code 68/356. The results are expressed as µIU/mL.

Expected values: Each laboratory should establish its own range of expected values for the population taken into consideration. To assess the expected reference range, a study was performed in 178 EDTA-plasma samples (89 subjects). Samples were collected from a fasting population formed of male (n = 49) and female (n = 40) apparently healthy blood donors of Caucasian race, and African-American, Hispanic and Asian origin, who meet the following inclusion criteria:
adult subjects, 18-65 years of age, with normal blood pressure and normal fasting glucose levels.
The following criteria prevented inclusion in this study:
age below 18 years; need for prescription medications; need for doctor-prescribed restricted diet; pregnancy; breast feeding; administration of oral contraceptives.

Blood was collected between 7:00 a.m. and 10:00 a.m. with the subjects either in an upright or supine position. Upright samples were collected when individuals sat down to have their blood withdrawn, after standing for 30 minutes; supine samples were collected after the individuals lay in supine position for at least 30 minutes.
The resulting intervals (5th-95th percentile) are the following:
4.4-46.1 µIU/mL (upright posture) and 2.8-39.9 µIU/mL (supine posture).

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® Direct Renin assay has been developed for the determination of the analyte in its intact and unaltered state. Degradation of the molecule or prorenin cryoactivation may affect final results.
– Although HAMA-neutralizing agents are added, extremely high HAMA (human anti-mouse antibodies) concentrations may occasionally influence results.
– Renin levels in paediatric age have not been investigated.
– No interference due to drug administration has been investigated.

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 500 mg/dL or triglycerides up to 3000 mg/dL.

Cross-reactions. The presence of the following potentially cross-reactive molecules in the assay showed the interference illustrated in the table below. The test was performed in accordance with the guidelines of Clinical and Laboratory Standards Institute (CLSI, USA), Document No. EP07-A2.

Compound	Spiked amount, µg/mL	% Cross-reactivity
$Beta_2$-microglobulin	50	– 7.0
Cathepsin D	1.5	– 6.9
Trypsin	1.6	– 4.2
Plasmin	100	0.8

In the absence of a standard prorenin preparation, cross-reactivity by prorenin was evaluated using two commercial preparations of recombinant prorenin and of recombinant renin. Two sets of five solutions with the same concentrations of the two molecules were prepared and the resulting samples (range: 0.78-12.5 ng/mL) were tested by direct renin immunoassays claiming no cross-reactivity by prorenin. The ratio of the signal emitted by each prorenin solution to the signal emitted by the corresponding renin solution is reported in the following table.

Immunoassay	CLIA direct renin test	IRMA direct renin test	Liaison Direct Renin
Prorenin-to-renin mean ratio	0.16	0.56	0.26

15.2. Precision

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	Control 1	Control 2
Number of determinations	20	20	20	20	20	20
Mean (µIU/mL)	15.1	33.8	82.2	258.0	27.1	99.0
Standard deviation (µIU/mL)	0.6	0.9	1.7	3.1	1.5	2.3
Coefficient of variation (%)	3.7	2.8	2.0	1.2	5.6	2.4
Min value (µIU/mL)	13.4	32.4	78.5	252.8	24.1	95.3
Max value (µIU/mL)	15.7	35.9	84.6	264.4	29.9	102.5

Reproducibility. Twenty replicates were performed in different days (one or two runs per day) with two different lots of integral per site 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	E	A	B	C	D	Control 1	Control 2
LOT No. 02							
Number of determinations	20	20	20	20	20	20	20
Mean (µIU/mL)	5.1	13.2	34.1	82.4	260.3	27.2	103.2
Standard deviation (µIU/mL)	0.5	1.6	1.9	4.3	12.3	1.9	4.4
Coefficient of variation (%)	10.0	12.4	5.7	5.2	4.7	6.9	4.2
Min value (µIU/mL)	4.1	11.4	30.8	74.3	241.6	24.3	93.7
Max value (µIU/mL)	5.9	17.2	38.4	88.7	278.8	30.4	111.0
LOT No. 03							
Number of determinations	20	20	20	20	20	20	20
Mean (µIU/mL)	5.3	13.1	34.7	83.5	266.7	27.3	102.9
Standard deviation (µIU/mL)	0.5	1.7	1.8	2.8	7.8	1.8	3.3
Coefficient of variation (%)	9.5	12.8	5.3	3.4	2.9	6.6	3.2
Min value (µIU/mL)	4.4	10.9	31.8	77.4	254.9	24.3	93.9
Max value (µIU/mL)	6.9	17.7	40.3	88.4	282.7	31.7	107.2
Inter-lot coefficient of variation (%)	2.7	0.6	1.2	0.9	1.7	0.4	0.2

Reproducibility - Site 2	A	B	C	D	Control 1	Control 2
LOT No. 01						
Number of determinations	20	20	20	20	20	20
Mean (µIU/mL)	18.8	38.4	79.1	228.6	27.5	97.2
Standard deviation (µIU/mL)	3.1	2.8	5.9	22.4	1.9	9.5
Coefficient of variation (%)	16.5	7.4	7.5	9.8	7.0	9.8
Min value (µIU/mL)	15.8	34.2	61.1	188.6	24.2	83.0
Max value (µIU/mL)	26.4	44.1	88.9	270.0	32.0	116.5
LOT No. 03						
Number of determinations	20	20	20	20	20	20
Mean (µIU/mL)	15.8	34.8	85.4	260.8	26.8	103.2
Standard deviation (µIU/mL)	2.7	2.1	5.2	26.5	2.8	7.5
Coefficient of variation (%)	17.1	6.1	6.1	10.2	10.6	7.3
Min value (µIU/mL)	12.9	32.3	75.2	214.5	22.1	91.0
Max value (µIU/mL)	22.7	38.4	93.7	306.2	31.7	116.3
Inter-lot coefficient of variation (%)	12.2	7.0	5.4	9.3	1.9	4.2

15.3. Linearity by dilution test

Plasma samples containing high renin concentrations were tested as such and after serially diluting with a renin-free plasma. Measured versus expected renin concentrations were analyzed by linear regression. The correlation coefficients (r) ranged from 0.999 to 1.000.

Dilution	Expected concentration, µIU/mL	Measured concentration, µIU/mL	% Recovery	Dilution	Expected concentration, µIU/mL	Measured concentration, µIU/mL	% Recovery
neat	–	333.4	–	neat	–	> 500.0	–
1:2	166.7	190.5	114.3	1:2	–	396.5	–
1:4	83.4	91.7	110.1	1:4	198.3	200.0	100.9
1:8	41.7	47.3	113.5	1:8	99.1	103.1	104.0
1:16	20.8	24.7	118.6	1:16	49.6	51.3	103.6
1:32	10.4	12.1	116.3	1:32	24.8	26.2	105.8

15.4. Trueness by recovery test

Two sets formed of a high- and a low- to normal-renin sample (samples X and Y in set 1, and 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 renin concentrations were analyzed by linear regression. The correlation coefficients (r) ranged from 0.996 to 1.000.

Set 1	Expected concentration, µIU/mL	Measured concentration, µIU/mL	% Recovery	Set 2	Expected concentration, µIU/mL	Measured concentration, µIU/mL	% Recovery
X neat	–	5.3	–	W neat	–	23.9	–
5:1	25.8	24.5	95.0	5:1	65.4	62.3	95.2
2:1	46.3	49.4	106.6	2:1	106.9	103.4	96.7
1:1	66.9	69.3	103.6	1:1	148.4	148.4	100.0
1:2	87.4	92.1	105.4	1:2	189.9	186.6	98.2
1:5	107.9	108.0	110.1	1:5	231.4	230.0	99.4
Y neat	–	128.4	–	Z neat	–	272.9	–

15.5. Carryover

The carryover effect was investigated by testing one renin-free plasma sample before and after four samples containing increasing renin concentrations. The results obtained demonstrate that no carryover is observed when using the LIAISON® Analyzer.

15.6. High-dose hook effect

The high-dose hook effect (HDH) was determined by addition of recombinant renin to a human plasma pool up to a maximum of 150,000 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 one high-concentration renin-spiked sample. The sample resulted in a calculated concentration value above the measuring 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 0.52 µIU/mL to 0.97 µIU/mL (as assessed by several assay runs, kit lots and instruments).

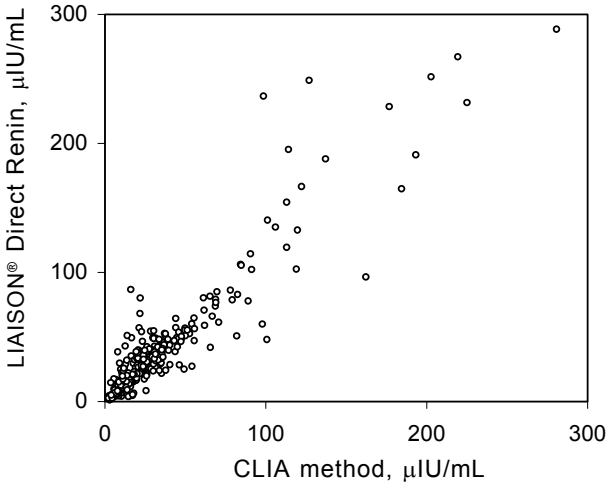
Analytical sensitivity, defined as the minimum detectable dose that can be distinguished from zero by two standard deviations (that is, two standard deviations above zero), ranges from 0.13 µIU/mL to 0.53 µIU/mL (as assessed by several assay runs, kit lots and instruments).

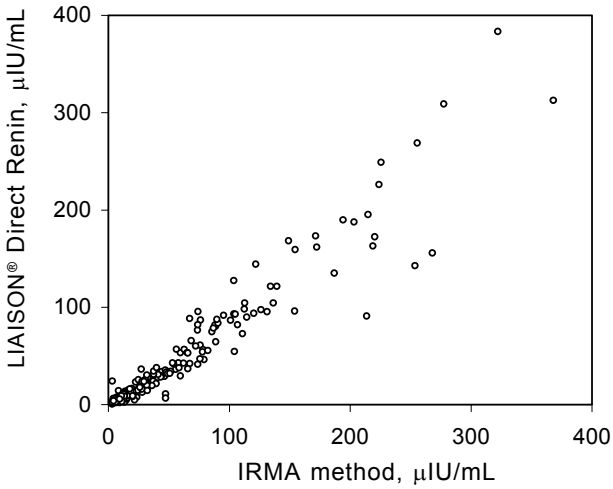
Functional sensitivity, defined as the concentration at which the between-assay coefficient of variation (CV) exceeds 20%, ranges from 1.60 µIU/mL to 1.96 µIU/mL (as assessed by several assay runs, kit lots and instruments).

15.8. Method comparison

The LIAISON® Direct Renin assay results were compared with those of two reference methods (CLIA and IRMA). The following correlations were obtained by linear regression analysis:
LIAISON® Direct Renin = 1.097 x reference CLIA method + 2.765. Correlation coefficient r = 0.927 (n = 325).
LIAISON® Direct Renin = 0.900 x reference IRMA method – 4.304. Correlation coefficient r = 0.957 (n = 212).

The nominal values of the LIAISON® Direct Renin calibrators have been assigned to reflect the accuracy data.





1. INTENDED USE

The LIAISON® Direct Renin controls (level 1 and level 2) are to be used in LIAISON® chemiluminescent immunoassays (CLIA) as a means of checking reliability of assay runs. The performance characteristics of LIAISON® Direct Renin controls have not been established in connection with any other assays or instrument platforms.

2. MATERIALS PROVIDED

4 x 2 vials

4 vials (4 x 2.0 mL) LIAISON® Control Direct Renin (level 1): recombinant human renin (active 340-amino acid protease produced in human embryonic kidney cells transfected with a human renin expression construct), phosphate buffer, bovine serum albumin, preservatives.
4 vials (4 x 2.0 mL) LIAISON® Control Direct Renin (level 2): recombinant human renin (active 340-amino acid protease produced in human embryonic kidney cells transfected with a human renin expression construct), phosphate buffer, bovine serum albumin, an inert blue dye, preservatives.
4 bar-coded labels for Control Direct Renin (level 1) and 4 bar-coded labels for Control Direct Renin (level 2).
The controls are provided lyophilized.

The range of concentrations of each control is reported on the corresponding vial label and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs. Each laboratory is responsible for adopting different limits to meet individual requirements.

3. WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic use.
- Controls are not kit lot specific and may be safely interchanged even from different lots.
- Observe the normal precautions required for handling all laboratory reagents.
- Disposal of all waste material should be in accordance with local guidelines.

4. SAFETY PRECAUTIONS

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

Do not pipette solutions by mouth.

Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses and disposable gloves. Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol. Any reagent spills should be washed with a 5% sodium hypochlorite solution and disposed of as though potentially infectious.

All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each Country.

5. STORAGE AND STABILITY

Do not leave the reconstituted controls at room temperature longer than the time required to process them on the LIAISON®.

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

6. PREPARATION OF REAGENTS

- Reconstitute the vial contents with 2.0 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 control must be transferred to a 12 x 75mm polystyrene tube. Affix the proper bar-coded label to the control tube and load on to the instrument specimen area. Each control solution allows nine tests to be performed.
- The minimum volume required is 350 µL (200 µL control + 150 µL dead volume).
- Keep the controls on board the instrument only for the amount of time required for quality control testing.
- After use, stopper transfer tubes promptly and store them at 2-8°C in an upright position.
- During handling, use appropriate precautions to avoid bacterial contamination of controls.

7. HANDLING

- Place the bar-coded transfer tubes in one of the patient racks on the LIAISON® Analyzer.
- Make sure that identical controls are not placed directly one after another.
- For further information of handling please refer to the LIAISON® Operator's Manual.

8. TARGET VALUES

The ranges of renin concentrations in the controls are printed on the vial labels. They have been established after taking into account run variability with respect to the stored master curve, in order to guarantee accuracy of analytical results and to obtain indications on stability or deterioration of reagents. If controls values lie repeatedly outside the expected ranges, the test has most probably been performed incorrectly.

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