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Changes: § 5
Deletions: §

LIAISON® Aldosterone (REF 310450)

1. INTENDED USE

The LIAISON® Aldosterone assay uses chemiluminescent immunoassay (CLIA) technology and is intended for the quantitative determination of Aldosterone in human serum, EDTA plasma and treated urine samples. Aldosterone measurements are intended for use in the diagnosis and treatment of primary aldosteronism (a disorder caused by excessive secretion of aldosterone by the adrenal gland), hypertension caused by primary aldosteronism, selective hypoaldosteronism, edematous states and other conditions of electrolyte imbalance.

The test has to be performed on the LIAISON® Analyzer Family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Aldosterone is a steroid hormone of molecular weight 360.4 daltons, the major mineralcorticoid secreted by the adrenal cortex. The role of aldosterone in metabolism is the control of sodium and potassium and therefore it regulates fluid volume. Aldosterone acts to decrease excretion of sodium and increase the excretion of potassium at the kidney, sweat glands, and salivary glands. Aldosterone also conserves sodium in the colon. In each of these tissues aldosterone works through binding at the mineralocorticoid receptors, and primarily at the cortical collecting ducts of the kidney. Regulation of sodium and potassium balance is accomplished through a complex set of hormones acting in several feedback loops. The renin-angiotensin systems (RAS), is the most important negative feedback loop for volume regulation. The RAS operates through a long feedback loop (involving changes in fluid volume) and a short feedback loop (with direct inhibition of renin secretion by angiotensin II). The other feedback loop that acts simultaneously is the control of serum potassium. These interacting feedback loops act in concert to set aldosterone concentrations to maintain homeostasis for volume, blood pressure, and potassium in response to external stimuli.
Aldosterone acts on the cortical collecting duct to increase the absorption of sodium and decreases the absorption of potassium. The resulting increase in fluid volume and blood pressure is sensed at the renin-secreting juxtaglomerular cells, which decrease the production of renin. With less renin, less angiotensin I is formed, and thereby angiotensin II levels are lowered. Lower levels of the acute stimulators of aldosterone secretion leads to decreased aldosterone synthesis and secretion. Aldosterone's role in the regulation of potassium homeostasis is also regulated primarily by a negative feedback loop. Increased potassium levels stimulate aldosterone production (aldosterone decreases reabsorption of potassium, thereby increasing potassium loss). The subsequent decrease in plasma potassium results in less potassium stimulation of the adrenal glomerulosa cells and lowers aldosterone synthesis and secretion.

Renin and aldosterone measurements are used in the investigation of patients with suspected:

- **Primary hyperaldosteronism** (PA, Conn's syndrome) is a disorder caused by excessive secretion of aldosterone by the adrenal gland, where high levels of circulating aldosterone are expected in the presence of low renin concentration or low plasmatic renin activity levels. Such inappropriately high aldosterone production (non-suppressible by sodium loading) causes cardiovascular damage, suppression of plasma renin, hypertension, sodium retention and potassium excretion that, if prolonged and severe, may lead to hypokalemia. The major causes of PA (>90% cases) are adrenal adenoma and unilateral or bilateral adrenal hyperplasia. Rare cases are due to the inherited condition of glucocorticoid-remediable aldosteronism.

- **Secondary hyperaldosteronism** is caused by disorders that activate the renin-angiotensin-aldosterone axis, resulting in excessive production of aldosterone (renovascular disease, salt depletion, potassium loading, cardiac failure with ascites, pregnancy, Bartter's syndrome).

- **Hypoaldosteronism** is a rare condition often due to primary adrenal failure, where plasma aldosterone has low concentrations in the presence of high concentrations of plasma renin. In hypoaldosteronism caused by low secretion of renin, low levels of plasma renin are expected in the presence of low levels of plasma aldosterone.

Because many factors (age, posture, sodium and potassium balance, time of day, menstrual cycle, etc.) influence the secretion of renin and aldosterone, evaluation of these hormones should be interpreted under strictly controlled conditions.

*(LIAISON®, LIAISON® XL and LIAISON® XS)

The assay of aldosterone may be carried out in serum, EDTA plasma and in 24-hour urine. From a clinical point of view it must be remembered that, while the 24-hour urinary measurement of 18-oxo-conjugate of aldosterone is an integrated reflection of the daily aldosterone secretion, the plasma values may reflect only a single point in time.

Since plasma aldosterone shows typical bursts, which follow a circadian rhythm, it might not be advisable to draw conclusions from a single determination. Plasma aldosterone measurement is used for acute studies (like circadian rhythms, postural changes, acute effect of drugs), the 24-hour urinary measurement helps determine the relationship between sodium excretion and aldosterone levels and may also be used to help to rule out high aldosterone secretion as a cause of another condition.

3. PRINCIPLE OF THE PROCEDURE

The method for the quantitative determination of the LIAISON® Aldosterone assay is a competitive assay that uses sheep monoclonal antibody for capture of the Aldosterone molecule.
The principle components of the test consist of magnetic particles (solid phase) coated with anti-sheep antibody that bind sheep anti-Aldosterone monoclonal antibody. An Aldosterone labeled conjugate containing an isoluminol derivative competes with Aldosterone from the calibrators, controls and patient samples.
During the first incubation, sample is incubated with a specific anti-Aldosterone monoclonal antibody. Following the 1st incubation the conjugate is added and competes with Aldosterone for an additional amount of time. After this 2nd incubation the unbound material is removed with a wash cycle. The starter reagents are then added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as relative light units (RLU) and is inversely proportional to the concentration of Aldosterone present in the calibrators, controls and patient samples.

4. MATERIALS PROVIDED

Reagent Integral

Magnetic Particles (2.4 mL)	SORB	Magnetic particles coated with anti-sheep antibody, sheep anti-aldosterone antibody in buffer containing Phosphate buffer/BSA, < 0.1% sodium azide.
Conjugate (4.5 mL)	CONJ	Proprietary polymer conjugated with aldosterone and an isoluminol derivative, BSA, phosphate buffer/Danazol, with ProClin® 300 and gentamicin sulfate as preservatives.
Assay Buffer (28.0 mL)	BUF AS	Phosphate buffer, EDTA, donkey and sheep serum with ProClin® 300 and gentamicin sulfate as a preservative and an inert yellow dye.
Number of tests		100

ProClin is a trademark of the Dow Chemical Company (Dow) or an affiliated company of Dow.

All reagents are supplied ready to use. The order of reagents reflects the layout of containers in the Reagent Integral.

Additonal components not on the Reagent Integral

Calibrator 1 2 x 3.0 mL	CAL 1	Human hormone free serum containing a low level of aldosterone, phosphate buffer, with ProClin® 300 and Gentamicin sulfate as preservatives.
Calibrator 2 2 x 3.0 mL	CAL 2	Human hormone free serum containing a high level of aldosterone, phosphate buffer, with ProClin® 300 and Gentamicin sulfate as preservatives.

The calibrator concentrations (ng/dL) are referenced to an in-house standard preparation.

Materials required but not provided (system related)

LIAISON® XL Analyzer	LIAISON® Analyzer	LIAISON® XS Analyzer
LIAISON® Wash/System Liquid (REF 319100)	LIAISON® Wash/System Liquid (REF 319100)	LIAISON® EASY Wash Buffer (REF 319301)
-	-	LIAISON® EASY System Liquid (REF 319302)
LIAISON® XL Waste Bags (REF X0025)	LIAISON® Waste Bags (REF 450003)	LIAISON® EASY Waste (REF X0054)
LIAISON® XL Cuvettes (REF X0016)	LIAISON® Module (REF 319130)	LIAISON® Cuvettes on Tray (REF X0053)
LIAISON® XL Starter Kit REF 319200) or LIAISON® EASY Starter Kit (REF 319300)	LIAISON® Starter Kit (REF 319102) or LIAISON® XL Starter Kit (REF 319200) or LIAISON® EASY Starter Kit (REF 319300)	LIAISON® EASY Starter Kit (REF 319300)
LIAISON® XL Disposable Tips (REF X0015) or LIAISON® Disposable Tips (REF X0055)	LIAISON® Cleaning Kit (REF 310990)	LIAISON® Disposable Tips (REF X0055)
-	LIAISON® Light Check 12 (REF 319150)	LIAISON® EASY Cleaning Tool (REF 310996)
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Additional Required Materials

LIAISON® Aldosterone Control Set (REF 310451)

Additional recommended materials:

0.2 N HCl
0.9% Saline
LIAISON® Aldo Neutralization Buffer (REF 310452)
LIAISON® Endocrinology Diluent (REF 319133)

5. WARNINGS AND PRECAUTIONS

FOR *IN VITRO* DIAGNOSTIC USE – Not for internal or external use in humans or animals.

General Safety:

- All specimens, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. Avoid contact with skin, eyes or mucous membranes. Follow good industrial hygiene practices during testing.
- 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 wearing lab coat, protective eye/face wear and disposable gloves.
- Wash hands thoroughly at the end of each assay.
- Avoid splashing or forming aerosols when handling, diluting or transferring specimens or reagents. Any reagent spill should be decontaminated with 10% bleach solution (containing 0.5% sodium hypochlorite) and disposed of as though potentially infectious.
- Waste materials should 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.
- Do not use kits or components beyond the expiration date given on the label.

Chemical Hazard and Safety Information: Reagents in this kit are classified in accordance with US OSHA Hazard Communication Standard; individual US State Right-to-Know laws; Canadian Centre for Occupational Health and Safety Controlled Products Regulations; and applicable European Union directives (see Material Safety Data Sheet for additional information).

Reagents Containing Human Source Material:

Warning – Treat as potentially infectious. Each serum/plasma donor unit used in the preparation of this product has been tested by an U.S. FDA approved method and found non-reactive for the presence of the antibody to Human Immunodeficiency Virus 1 and 2 (HIV 1/2), the Hepatitis B surface antigen (HBsAg), and the antibody to Hepatitis C (HCV). While these methods are highly accurate, they do not guarantee that all infected units will be detected. This product may also contain other human source diseases for which there is no approved test. Because no known test method can offer complete assurance that HIV, Hepatitis B Virus (HBV) and HCV or other infectious agents are absent, all products containing human source material should be handled following universal precautions; and as applicable in accordance with good laboratory practices as described in the Centers for Disease Control and the National Institutes of Health current manual, Biosafety in Microbiological and Biomedical Laboratories (BMBL); or the World Health Organization current edition, Laboratory Biosafety Manual.

GHS/CLP:

	ProClin®	Sodium Azide
CAS No.:	55965-84-9	26628-22-8
Reagents:	CONJ CAL 1 CAL 2 BUF AS	SORB
Classification:	Skin sensitization , Category 1 Aquatic Chronic, Category 3	None required
Signal Word:	Warning	None required
Pictogram:	 GHS07 – Exclamation mark	None required
Hazard Statements:	H317 – May cause an allergic skin reaction. H412 – Harmful to aquatic life with long lasting effects.	None required
Precautionary Statements:	P261 – Avoid breathing mist or spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P273 – Avoid release to the environment. P280 – Wear protective gloves and clothing, and eye protection.	None required

Reagents Containing Sodium Azide: Sodium azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts," in the Manual Guide-Safety Management No. CDC-22 issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.

6. REAGENT INTEGRAL PREPARATION

Please note the following important reagent handling precautions:

6.1 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 color 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 been 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.

6.2 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 re-suspension 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.

6.3 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 and LIAISON® XS Analyzer

- LIAISON® XL Analyzer and LIAISON® XS 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.

7. REAGENT INTEGRAL STORAGE AND STABILITY

Upon receipt, the Reagent Integral must be stored in an upright position to facilitate resuspension of magnetic particles. See Reagent Integral Preparation for resuspension instructions. When the Reagent Integral is stored sealed, the reagents are stable at 2-8°C up to the expiration date. Do not freeze. The Reagent Integral must not be used past the expiration date indicated on the kit and Reagent Integral labels. After removing the seals, the Reagent Integral is stable for 6 weeks when stored at 2-8°C in a refrigerator or on board the LIAISON® Analyzer.

8. SPECIMEN COLLECTION AND PREPARATION

Human serum, EDTA-plasma or 24-hour Urine samples may be used in this assay.

Serum and EDTA Plasma

Blood should be collected aseptically by venipuncture, noting time of day and position of patient (supine or upright). Samples should be allowed to clot, and the serum separated from the clot as soon as possible. EDTA plasma samples should be centrifuged in a non-refrigerated centrifuge; remove the EDTA plasma from the cells immediately after centrifugation.

Samples having particulate matter, turbidity, lipemia or erythrocyte debris may require clarification by filtration or centrifugation before testing. Grossly hemolyzed, icteric or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination are not recommended and should not be tested.

Check for and remove air bubbles before assaying. **If the assay is performed within 5 days of sample collection, the samples may be kept at 2-8°C; otherwise they should be dispensed in aliquots and stored deep-frozen (-20°C or below).** If samples are stored frozen, mix thawed samples well before testing. Self-defrosting freezers are not recommended for sample storage. Samples are stable for 4 weeks at -20°C. Samples are stable through 3 freeze thaw cycles.

Urine samples

Collect 24-hour urine, keeping the specimen refrigerated during collection, measure and record the volume. Add 1 g boric acid/100 mL urine. Mix well before withdrawing an aliquot to be assayed. Borate stabilized urine samples are stable for 5 days when stored at 2-8°C or for 4 weeks when stored at -20°C. Samples are stable through 3 freeze thaw cycles.

Urine hydrolysis

Aldosterone can be assayed in urine samples after acid hydrolysis of aldosterone 18-glucuronide.
- Label a glass or polypropylene tube for each urine sample.
- Add 1 part urine and 2 parts 0.2 N HCl (not supplied). Mix thoroughly by vortex or rotator.

Example (100 µL urine and 200 µL 0.2 N HCl).

- Cap tubes and incubate overnight for 18 hours at 30°C.

Urine Neutralization

After hydrolysis, the urine sample must be neutralized before testing on the LIAISON® Aldosterone assay.
- Label a glass or polypropylene tube for each urine sample
- Add 1 part hydrolyzed urine and 4 parts LIAISON® Aldo Neutralization Buffer, mix well.

Example (100 µL hydrolyzed urine and 400 µL LIAISON® Aldo Neutralization Buffer).

Check for and remove air bubbles before assaying. Hydrolyzed and neutralized urine preps are stable for 8 weeks when stored at 2-8°C or -20°C. If samples are stored frozen, mix thawed samples well before testing. Samples should not be repeatedly frozen and thawed. Self-defrosting freezers are not recommended for sample storage.

The minimum volume required for either serum or neutralized urine testing on the LIAISON® Analyzer is 250 µL per specimen [100 µL specimen for testing + 150 µL dead volume (volume left at the bottom of the aliquot tube which the instrument cannot aspirate)]**.**

9. Calibrators Level 1 and Level 2

The LIAISON® Aldosterone calibrators are liquid and ready to use. Upon receipt, the calibrators must be stored at 2-8°C in an upright position. Unopened calibrators are stable at 2-8°C up to the expiry date indicated on the kit and calibrator labels. Calibrators should be equilibrated to room temperature and mixed thoroughly by gentle inversion. Calibrate the assay as described in the Operator's manual. Once opened remaining liquid calibrators can be stored at 2-8°C for 6 weeks.

LIAISON® Analyzer:

Transfer the vial to the LIAISON® Analyzer "L" rack and place onto the LIAISON® Analyzer.

LIAISON® XL Analyzer and LIAISON® XS Analyzer:
Transfer the vial to the LIAISON® XL and LIAISON® XS Analyzer "L" rack and place on the LIAISON® XL Analyzer or LIAISON® XS Analyzer.

Calibrator and reagent integral lot number are lot specific. Do not use calibrators matched with a different reagent lot in the same assay.

10. CALIBRATION

Assay of calibrators contained in the Reagent Integral allows the analyzer to recalibrate the stored master curve, as indicated via the bar codes on the Reagent Integral label. Test of assay specific calibrators allows the detected relative light units (RLU) values to adjust the assigned master curve. Each calibration solution allows 6 calibrations to be performed.
Recalibration in triplicate is mandatory whenever at least 1 of the following conditions occurs:
- A new lot of Reagent Integral or of Starter Kit is used.
- The previous calibration was performed more than 14 days before.
- The analyzer has been serviced.
- The values of the recommended LIAISON® Aldosterone Controls lie outside the expected ranges.

Refer to the analyzer operator's manual or LIAISON® Quick Guide for calibration instructions.

Measuring range: The LIAISON® Aldosterone assay measures between 0.97 and 100 ng/dL.
The lowest reportable value is 0.97 ng/dL. Values below 0.97 ng/dL should be reported as < 0.97 ng/dL. The highest reportable value without dilution is 100 ng/dL.

Serum or EDTA plasma samples that read above the assay range may be diluted with the LIAISON® Endocrinology Diluent (REF 319133) and retested.
Suggested dilution: 1 part sample and 9 parts Endocrinology Diluent.

Urine samples that read above the assay range may be diluted with either deionized water or 0.9% saline and retested.
Suggested dilution: 1 part hydrolyzed/neutralized urine and 9 parts deionized water or 0.9% saline.

11. ASSAY PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the LIAISON® Analyzer.

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 and LIAISON® XS 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.

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

12. QUALITY CONTROL

Quality control is performed once per day of use or in accordance with local, state and/or federal regulations or accreditation requirements and your laboratory's quality control procedures. It is recommended that the user refer to CLSI C24-A3, and 42 CFR 493.1256 (c) for guidance on appropriate quality control practices.

LIAISON® controls should be equilibrated to room temperature, and mixed thoroughly before use either by rotation or gentle inversion.

LIAISON® controls should be run in singlicate to monitor the assay performance. If control values lie within the expected ranges provided on the certificate of analysis, the test is valid. If control values lie outside the expected ranges, the test is invalid and patient results cannot be reported. Assay calibration should be performed if a control failure is observed and controls and patient specimens must be repeated.

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

The range of concentrations of each control is reported on the certificate of analysis and indicates the limits established by DiaSorin for control values that can be obtained in reliable assay runs.

13. INTERPRETATION OF RESULTS

The LIAISON® Analyzer automatically calculates the concentration of aldosterone in the serum samples. This concentration is expressed in ng/dL.

To convert results to pg/mL: 1 ng/dL = 10 pg/mL. To convert results to nmol/L: 1 ng/dL = 0.0277 nmol/L.

For Urine: The result read from the calibration curve and provided on the Analyzer printout in ng/dL must be multiplied by a correction factor of 15 to account for the dilution of hydrolyzed and neutralized urine.

In addition, the users must record the total volume of the 24-hour urine sample in order to report the total mass of aldosterone in µg/24 hours.

To calculate urine aldosterone in µg/24 hours, user shall use the following formula:
corrected aldosterone concentration (result from analyzer in ng/dL x 15) x 24 hour urine volume (in mL) x 10^{-5}.

14. LIMITATIONS OF THE PROCEDURE

1. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.
2. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
3. Grossly hemolyzed, icteric or lipemic samples, as well as samples containing particulate matter or exhibiting obvious microbial contamination, are not recommended and should not be tested.
4. Bacterial contamination of samples may affect the test results.
5. Do not heat inactivate serum, EDTA plasma or urine samples.

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. EXPECTED VALUES

It is recommended that each laboratory establish its own range of expected values for the population taken into consideration.
To assess the expected reference range, a study was performed with 126 serum and EDTA plasma samples (126 subjects). Samples were collected from a fasting population formed of male and female apparently healthy blood donors who meet the following inclusion criteria: Adult subjects 21 - 65 years of age, with normal blood pressure and normal fasting glucose levels.
The following criteria prevented inclusion in this study: Age below 21 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 from individuals after standing for at least 30 minutes.
Supine samples were collected after the individuals lay in supine position for at least 30 minutes.
Based on the 95% confidence interval, the following values were established following CLSI guideline C28-A3. The following values were obtained:

Population (126)	Median Aldosterone (ng/dL)	Observed Range (ng/dL) 2.5^{th} to 97.5^{th} Percentile
Upright (Serum)	9.80	2.52 – 39.2
Supine (Serum)	6.76	1.76 - 23.2
Upright (EDTA)	8.91	2.21 – 35.3
Supine (EDTA)	6.42	1.17 – 23.6

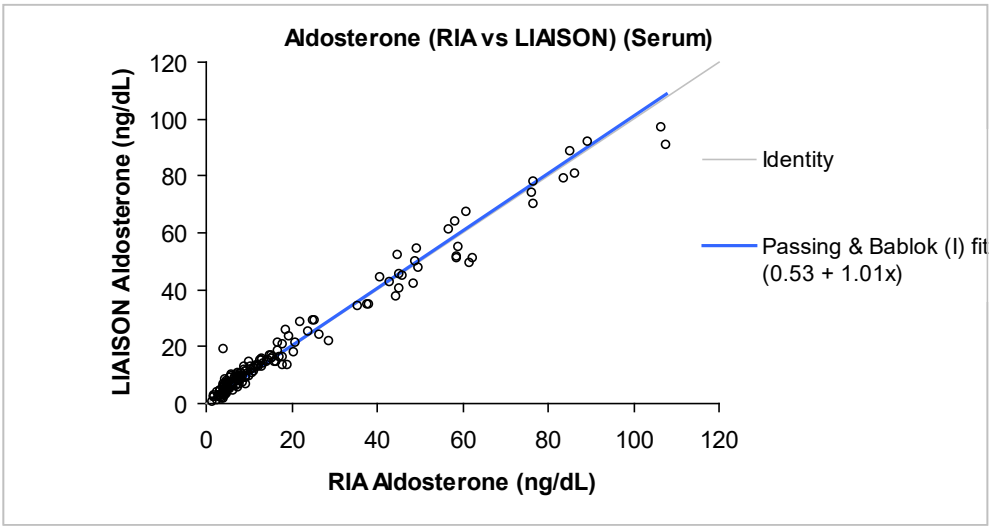
To assess the expected urine aldosterone reference range; a study was performed with 91 urine samples collected over a 24 hour period from apparently healthy subjects with a normal blood pressure (diastolic < 85 mmHg).

Population (91)	Median Aldosterone (µg/day)	Observed Range (µg/day) 2.5^{th} to 97.5^{th} Percentile
Urine (24 hour)	5.53	1.19 – 28.1

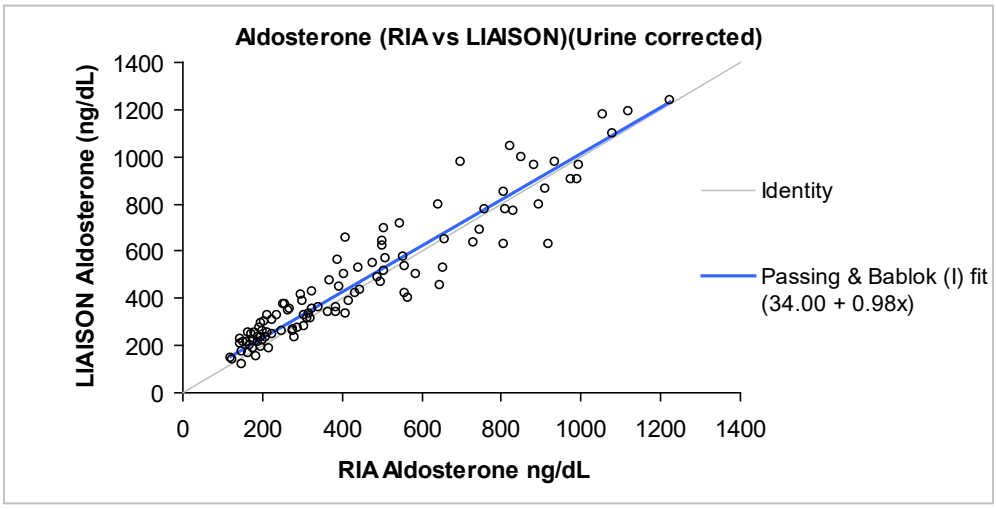
16. SPECIFIC PERFORMANCE CHARACTERISTICS

16.1 Patient Correlation/Method Comparison:

A total of 155 serum samples or prepared samples spanning the assay range, were tested by the LIAISON® Aldosterone assay and by a manual RIA method following CLSI EP9, and yielded the following Passing & Bablok regression analysis: LIAISON® Aldosterone = 1.01(Reference Method) + 0.53; R^2 = 0.98



A total of 104 urine samples spanning the assay range were tested by the LIAISON® Aldosterone assay and by a manual RIA method following CLSI EP9. Urine sample results were corrected for dilution as required by each respective assays' Instructions for Use. Reference RIA method: result x 10. LIAISON® Aldosterone result x 15.
The resulting Passing & Bablok regression equation is: LIAISON® Aldosterone = 0.98(Reference Method) + 34, R^2 = 0.90.



16.2 Precision

2 kit controls, 6 serum samples and 3 urine samples all containing concentrations of aldosterone prepared to span the range of the assay were tested twice per day in duplicate, over 20 operating days using 2 reagent lots at 2 external sites and DiaSorin. The testing was performed according to CLSI EP5-A2.

Sample ID	Mean (ng/dL)	Within Run		Total/Across Lots/Across Sites	
		SD	%CV	SD	%CV
Kit Control Level 1	6.8	0.24	3.5%	0.65	9.5%
Kit Control Level 2	28.8	0.53	1.8%	1.61	5.6%
Aldo S1	5.9	0.25	4.2%	0.62	10.5%
Aldo S2	8.8	0.27	3.1%	0.79	9.0%
Aldo S3	18.5	0.42	2.3%	1.27	6.9%
Aldo S4	29.8	0.78	2.6%	2.05	6.9%
Aldo S5	50.4	1.16	2.3%	2.92	5.8%
Aldo S6	82.6	1.76	2.1%	5.21	6.3%
Aldo U1	7.4	0.26	3.6%	0.72	9.8%
Aldo U2	44.1	1.24	2.8%	3.87	8.8%
Aldo U3	76.3	1.91	2.5%	6.58	8.6%

LIAISON® XS Analyzer: 2 kit controls, 6 serum samples and 3 urine samples were prepared and tested at DiaSorin Inc. once per day in replicates of 6, over 5 operating days on 3 LIAISON® XS Analyzers, using 1 reagent lot of the LIAISON® Aldosterone assay. The testing was performed according to CLSI EP15-A3.

Sample ID	Mean (ng/dL)	Within Run		Total	
		SD	%CV	SD	%CV
Kit Control Level 1	6.19	0.42	6.8%	0.56	9.1%
Kit Control Level 2	26.0	0.63	2.4%	1.09	4.2%
Aldo S1	5.93	0.37	6.2%	0.58	9.7%
Aldo S2	8.06	0.36	4.5%	0.60	7.5%
Aldo S3	24.9	0.42	1.7%	0.86	3.4%
Aldo S4	30.1	0.42	1.4%	1.03	3.4%
Aldo S5	48.9	0.92	1.9%	2.39	4.9%
Aldo S6	87.3	1.38	1.6%	4.21	4.8%
Aldo U1	11.7	0.66	5.6%	0.92	7.9%
Aldo U2	46.2	1.24	2.7%	2.51	5.4%
Aldo U3	71.5	1.62	2.3%	4.12	5.8%

16.3 LoD (Limit of Detection)

Following the method from CLSI EP17-A2, the limit of detection for the LIAISON® Aldosterone assay for serum is 1.45 ng/dL.
The limit of detection for the LIAISON® Aldosterone assay for urine is 2.0 ng/dL (2.0*15 =30 ng/dL).

16.4 LoB (Limit of Blank)

Following the method from CLSI EP17-A2, the limit of blank for the LIAISON® Aldosterone assay for serum is ≤ 0.97 ng/dL.
The limit of blank for the LIAISON® Aldosterone assay for urine is ≤ 1.26 ng/dL (1.26*15 = 18.9 ng/dL).

*Limit of Blank, or the highest value likely to be observed with a sample containing no analyte, replaces the term “analytical sensitivity”.

16.5 LoQ (Limit of Quantitation)

Following the method from CLSI EP17-A2, the limit of quantitation for the LIAISON® Aldosterone assay for serum is 1.91 ng/dL.
The limit of quantitation for the LIAISON® Aldosterone assay for urine is 2.8 ng/dL (2.8*15 = 42 ng/dL).

16.6 Trueness Dilution Test (Linearity)
1 serum, 1 EDTA plasma and 1 urine sample were diluted and analyzed by the LIAISON® Aldosterone assay following CLSI EP6-A. The results were analyzed by a linear regression of Observed Aldosterone concentration versus Expected Aldosterone Concentration.

The resulting equation for serum sample is: Observed LIAISON® Aldosterone = 0.994(Expected) + 0.71, R^2=0.99
The resulting equation for EDTA plasma sample is: Observed LIAISON® Aldosterone = 1.01(Expected) + 1.43, R^2=0.99
The resulting equation for urine sample is: Observed LIAISON® Aldosterone = 0.996(Expected) + 0.69, R^2=0.99

16.7 Recovery
5 high concentrations each of serum and urine samples and 5 low concentrations each of serum and urine samples were analyzed neat. Recovery samples were then prepared by mixing defined ratios of the high and low samples and tested in replicates of 5. The mean of the 5 replicates are provided in the table below.

Serum Samples	Defined Concentration	Expected ng/dL	Observed ng/dL	% Recovery
High Sample 1 (HS1)	58.0			
2 HS1 : 1 LS1		40.9	37.9	93%
1 HS1 : 1 LS1		32.1	29.2	91%
1 HS1 : 2 LS1		23.3	21.1	91%
Low Sample 1 (LS1)	6.1			
High Sample 2 (HS2)	69.6			
2 HS2 : 1 LS2		49.3	48.6	99%
1 HS2 : 1 LS2		38.9	39.1	101%
1 HS2 : 2 LS2		28.4	28.3	100%
Low Sample 2 (LS2)	8.1			
High Sample 3 (HS3)	89.1			
2 HS3 : 1 LS3		62.8	63.5	101%
1 HS3 : 1 LS3		49.2	50.0	102%
1 HS3 : 2 LS3		35.7	37.2	104%
Low Sample 3 (LS3)	9.3			
High Sample 4 (HS4)	91.9			
2 HS4 : 1 LS4		64.0	63.0	98%
1 HS4 : 1 LS4		49.6	52.2	105%
1 HS4 : 2 LS4		35.2	35.6	101%
Low Sample 4 (LS4)	7.3			
High Sample 5 (HS5)	86.6			
2 HS5 : 1 LS5		60.2	61.7	103%
1 HS5 : 1 LS5		46.6	47.5	102%
1 HS5 : 2 LS5		33.0	33.7	102%
Low Sample 5 (LS5)	6.5			
		Mean Recovery		99.5%

Urine Samples	Defined Concentration	Expected ng/dL	Observed ng/dL	% Recovery
High Sample 1 (HS1)	97.4			
2 HS1 : 1 LS1		66.7	62.9	94%
1 HS1 : 1 LS1		50.9	48.2	95%
1 HS1 : 2 LS1		35.1	36.7	105%
Low Sample 1 (LS1)	4.4			
High Sample 2 (HS2)	87.9			
2 HS2 : 1 LS2		60.2	57.4	95%
1 HS2 : 1 LS2		46.0	43.8	95%
1 HS2 : 2 LS2		31.7	32.4	102%
Low Sample 2 (LS2)	4.0			
High Sample 3 (HS3)	77.8			
2 HS3 : 1 LS3		54.0	51.6	95%
1 HS3 : 1 LS3		41.8	41.4	99%
1 HS3 : 2 LS3		29.6	28.7	97%
Low Sample 3 (LS3)	5.8			
High Sample 4 (HS4)	70.8			
2 HS4 : 1 LS4		49.4	46.3	94%
1 HS4 : 1 LS4		38.3	37.6	98%
1 HS4 : 2 LS4		27.3	27.2	100%
Low Sample 4 (LS4)	5.8			
High Sample 5 (HS5)	72.9			
2 HS5 : 1 LS5		50.5	49.6	98%
1 HS5 : 1 LS5		39.0	38.8	99%
1 HS5 : 2 LS5		27.5	27.5	100%
Low Sample 5 (LS5)	5.1			
		Mean Recovery		97.8%

16.8 Interfering substances

Controlled studies of potentially interfering substances at 2 Aldosterone levels in serum (15 and 30 ng/dL) and urine (5 and 15 ng/dL) showed no interference in the LIAISON® Aldosterone assay at the highest concentration for each substance listed below. The testing was based on CLSI-EP7-A2

Substance/Drug	Concentration Tested		Substance/Drug	Concentration Tested	
	Serum	Urine		Serum	Urine
Bilirubin (conjugated)	40 mg/dL	40 mg/dL	Propranolol hydrochloride	2.3 µg/mL	2.3 µg/mL
Bilirubin (unconj)	40 mg/dL	N/A	Metoprolol	12.8 µg/mL	12.8 µg/mL
Hemoglobin	600 mg/dL	600 mg/dL	Triamterene	8.86 µg/mL	8.86 µg/mL
Triglycerides	3000 mg/dL	3000 mg/dL	Spironolactone	0.6 µg/mL	0.6 µg/mL
Total protein	12 g/dL	12 g/dL	Tetracycline	15.1 µg/mL	15.1 µg/mL
Cholesterol	500 mg/dL	500 mg/dL	Amlodipine besylate	13.9 µg/dL	13.9 µg/dL
Creatinine	5 mg/dL	500 mg/dL	Nifedipine	40 µg/dL	439 µg/mL
Glucose	1 g/dL	1 g/dL	Verapamil	216 µg/dL	2.37 mg/mL
Ascorbic Acid	6 mg/dL	200 mg/dL	Furosemide	59.9 µg/mL	59.9 µg/mL
Urea	N/A	4 g/dL	Eplerenone	19.9 µg/mL	19.9 µg/mL
Boric Acid	N/A	2 g/dL	Enalapril	42.4 µg/dL	466 µg/mL
Acetic Acid	N/A	2%	Lisinopril	32.7 µg/dL	32.7 µg/dL
Acetaminophen	20 mg/dL	20 mg/dL	Losartan potassium	2.25 µg/mL	2.47 mg/mL
Acetylsalicylic acid	65.2 mg/dL	65.2 mg/dL	Valsartan	11 µg/mL	11 µg/mL
Salicylic acid	59.9 mg/dL	59.9 mg/dL	Hydrochlorothiazide (HCTZ)	6.0 µg/mL	6.0 µg/mL
Valproic Acid	57.6 mg/dL	57.6 mg/dL	Uric Acid	N/A	100 mg/dL
Tartaric Acid	N/A	1 g/dL			

16.9 Cross-reactants

Controlled studies of potentially Cross-reactive substances in serum and urine samples were performed on the LIAISON® Aldosterone assay with the at the concentrations listed below. All substances showed < 0.02% cross reactivity. The testing was based on CLSI-EP7-A2.

Substance/Drug	Concentration Tested	
	Serum	Urine
Androstendione	100 ng/mL	1000 ng/mL
Androsterone	1000 ng/mL	10000 ng/mL
Corticosterone	1000 ng/mL	1000 ng/mL
18-OH-Corticosterone	1000 ng/mL	1000 ngmL
Cortisol	1000 ng/mL	2000 ng/mL
Cortisone	2000 ng/mL	2000 ng/mL
11-Deoxycorticosterone	1000 ng/mL	1000 ng/mL
11-Deoxycortisol	1000 ng/mL	1000 ng/mL
Dexamethasone	2000 ng/mL	2000 ng/mL
DHEA	1000 ng/mL	10000 ng/mL
Estradiol	1000 ng/mL	1000 ng/mL
Estriol	100 ng/mL	1000 ng/mL
Estrone	100 ng/mL	1000 ng/mL
Fludrocortisone	2000 ng/mL	2000 ng/mL
Prazosin HCl	12000 ng/mL	12000 ng/ml
Prednisone	1000 ng/mL	1000 ng/mL
Prednisolone	1000 ng/mL	1000 ng/mL
Pregnenolone	1000 ng/mL	1000 ng/mL
Progesterone	1000 ng/mL	1000 ng/mL
17 alpha Hydroxyprogesterone	1000 ng/mL	1000 ng/mL
Testosterone	1000 ng/mL	2000 ng/mL
Spironolactone	1000 ng/mL	1000 ng/mL

16.10 High Dose Hook Effect

No High dose hook effect was observed for aldosterone concentrations in serum and urine up to 1000 ng/dL.

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