Comparison of the Chemiluminescence Immunoassay LIAISON® with the Radioimmunoassay for Aldosterone and Renin Measurement

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Abstract: Determination of renin plasma levels is useful in the diagnosis of hypertension and in the therapeutic follow-up of hypertensive patients. Plasmatic concentration of renin decreases in patients with hypertension due to a primary hyperaldosteronism, contrary to renovascular hypertension where concentrations of renin and aldosterone are both elevated. Blood samples (serum, EDTA plasma) were analysed using two different chemiluminiscent methods CLIA LIAISON® and radioimmunoassay for aldosterone (IMMUNOTECH Beckman Coulter) and renin (Cisbio Bioassay) measurements were compared. We used both methods to ascertain the correlation between serum vs. EDTA plasma levels of aldosterone (RIA, CLIA) and renin (IRMA, CLIA) and to compare aldosterone to renin ratios for CLIA and for radioimmunoassay: serum aldosterone to plasma renin and plasma aldosterone to plasma renin. We compared serum aldosterone CLIA vs. RIA ($r_p=0.933, P<0.001$) and plasma renin determined using CLIA vs. IRMA ($r_p=0.965, P=0.062$). Furthermore, we used both methods to establish the correlation between the serum vs. plasma levels of aldosterone: RIA ($r_p=0.980, P<0.001$); CLIA ($r_p=0.994, P=0.353$) and serum vs. plasma levels of renin: IRMA ($r_p=0.948$, $P<0.001$).

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P<0.001); CLIA ($r_p=0.921$, $P=0.011$). Aldosterone (serum, plasma) to plasmatic renin ratios for CLIA ($r_p=0.999$, $P=0.286$) and for radioimmunoassay ($r_p=0.992$, $P=0.025$). Our data demonstrate that renin and aldosterone concentrations obtained using CLIA correlate with renin and aldosterone concentrations using radioimmunoassay methods. Correlation coefficients of pair results ranged from 0.921 to 0.994. Aldosterone (serum, EDTA plasma) to plasmatic renin ratios are comparable and any of them can be used with no significant differences found.

**Introduction**

Primary hyperaldosteronism (PH) is nowadays the most frequent form of secondary endocrine-mediated hypertension. Primary hyperaldosteronism (PH) diagnostics based on the determination of aldosterone levels, plasma renin activity and their ratio has become obsolete. Instead of the demanding determination of plasma renin activity, direct renin measurement is now performed (Wedatilake et al., 2011; Jensen et al., 2014; Douillard et al., 2016). Renin also plays a role in the release of aldosterone, a hormone that also controls the body natrium and water balance. Measurement of serum aldosterone in conjunction with plasma renin and their ratio are used clinically to differentiate between primary and secondary hyperaldosteronism (Trenkel et al., 2002; Reincke et al., 2003; Barigou et al., 2015).

At our laboratory, we used to perform determination of serum levels of aldosterone and plasma renin by radioimmunoassay. Our tests on two analytical systems were mainly focused on comparing the results of serum aldosterone (RIA vs. CLIA) and plasma renin (IRMA vs. CLIA). Subsequently, we tested how much the choice of material (serum, plasma) affects the results of the analysis of aldosterone and renin (CLIA vs. RIA or IRMA). The aldosterone and renin concentrations dependency on sample material (serum and EDTA plasma) was evaluated using both analytical systems. Our task was to compare the CLIA LIAISON® technology with a radioimmunoassay method in samples of serum and EDTA plasma. Parallel sample testing was conducted using both analytical methods, using the LIAISON® automated immunoanalyzer (CLIA) and the STRATEC SR 300 automated immunoanalyzer (RIA, IRMA). For both analytical systems (CLIA, RIA or IRMA) we compared the ratios: serum aldosterone to plasmatic renin and plasmatic aldosterone to plasmatic renin.

Plasma renin activity (angiotensin I) was not determined in tested samples.

**Material and Methods**

**Material**

From single blood draw we prepared two sets of identical serum samples and EDTA plasma samples for parallel determination of aldosterone (CLIA and RIA) and two sets of identical EDTA plasma samples for renin determination (CLIA and IRMA). The samples were stored at –20 °C until the measurements, but not longer than one month. Fresh samples analysed by routine method were chosen to cover low to high values along the calibration curve. Icteric and lipemic samples as well as samples...
with results out of calibration range for any of analytes (renin, aldosterone) were excluded. Samples tested were obtained from men and women aged 20–60 years. Samples come from both inpatient and outpatient hospital populations after appropriate diet. All were taken in sitting position after at least 30 minutes long resting from fasting patients in morning hours. Samples were transported in 5–15 °C and centrifuged in room temperature within one hour at 3,000 rpm for 10 min. Samples were stored at −20 °C for maximum of 1-month prior analysis.

Methods

Aldosterone and renin were determined by two methods, in parallel CLIA and radioimmunoassay. Aldosterone and renin were determined according to the instructions for use given by producers.

RIA procedure of aldosterone

Aldosterone was determined using the ALDOSTERONE RIA kit (Beckman Coulter, France). The kit is intended for direct quantitative determination of aldosterone in serum and EDTA plasma for in vitro diagnostics. Aldosterone determination is a competitive radioimmunoassay (RIA, a radionuclide marked ¹²⁵I-aldosterone). A total of 50 µl plasma or serum and 500 µl of the tracer (¹²⁵I-aldosterone) are incubated for 3 hours at 18–25 °C with a solid phase anti-aldosterone monoclonal antibody. At the end of the incubation the unbound material is removed, the concentration of aldosterone is calculated by extrapolation of a spline curve with six calibrators.

Performance characteristics of aldosterone RIA assays: limit of quantification (LOQ) 6.0 ng/l, intra-assay: CV (coefficient of variation) ≤ 9.5%, inter-assay: CV ≤ 10.4%, measurement range 6.0–2,000 ng/l.

CLIA procedure of aldosterone

Chemiluminescence tests provided by the LIAISON® Aldosterone assay (DiaSorin, USA). The kit is intended for quantitative determination of aldosterone in human serum and EDTA plasma for the purposes of in vitro diagnostics. The method for the quantitative determination of the aldosterone assay is a competitive assay that uses sheep monoclonal antibody to capture the aldosterone molecule. The principle components of the test consist of magnetic particles (solid phase) coated with anti-sheep antibody that binds sheep anti-aldosterone monoclonal antibody. An aldosterone labelled conjugate containing an isoluminol derivative competes with aldosterone from the calibrators, controls and patient samples. During the first incubation (55 minutes), sample is incubated with a specific anti-aldosterone monoclonal antibody. Following this incubation, the conjugate is added and competes with aldosterone for an additional amount of time. After the second 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. The final values of aldosterone are calculated with a two-point working curve adjusted against a stored master curve. The analyser automatically calculates renin concentrations for the unknown samples expressed as ng/l and grades the results.

Performance characteristics of aldosterone CLIA assays: limit of quantification (LOQ) 19.1 ng/l, intra-assay: CV = 1.8–4.2%, inter-assay: CV = 5.6–10.5%, measurement range 9.7–1,000 ng/l.

**IRMA procedure of renin**
Renin was measured using the RENIN III GENERATION kit (Cisbio Bioassay, France). The kit is intended for quantitative determination of direct renin in EDTA plasma for *in vitro* diagnostics. The principle is based on non-competitive immunoradiometric assay (IRMA, two anti-renin monoclonal mouse antibodies (MAb); 1<sup>st</sup> MAb is fixed to the vial wall (specific for renin, prorenin); 2<sup>nd</sup> MAb is marked with radionuclide ¹²⁵I (specific for renin). A total of 300 µl plasma and 100 µl of the tracer (2<sup>nd</sup> MAb-¹²⁵I) are incubated for 3 hours at 18–25 °C with a solid phase monoclonal specific antibody for renin (1<sup>st</sup> MAb). After incubation, the unbound material is removed with a wash cycle, the concentration of renin is calculated by extrapolation of a spline curve with six calibrators.

Performance characteristics of renin IRMA assays: limit of quantification (LOQ) 1.0 ng/l, intra-assay: CV ≤ 3.6%, inter-assay: CV ≤ 5.0%, measurement range 2.5–320.0 ng/l.

**CLIA procedure of renin**
The Liaison<sup>®</sup> Direct Renin kit (DiaSorin, Italy) is intended for quantitative determination of renin concentration in human EDTA plasma samples for the purposes of *in vitro* diagnostics. The method for the quantitative determination of renin is a sandwich CLIA. A specific mouse monoclonal antibody is coated on the magnetic particles (solid phase), that recognizes both renin and prorenin; another mouse monoclonal antibody (specific for renin) is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the incubation (38 minutes), 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, patient samples or controls. The final values of plasma renin concentration are calculated with a two-point working curve.
adjusted against a stored master curve. The analyser automatically calculates renin concentrations for the unknown samples expressed as pg/ml and grades the results.

Performance characteristics of renin CLIA assays: limit of quantification (LOQ) 0.3 ng/l, intra-assay: CV ≤ 2.38%, inter-assay: CV ≤ 7.31%, measurement range 2.5–316.5 ng/l.

**Scheme of experiments of CLIA vs. RIA or IRMA assays**
1) CLIA vs. IRMA of plasma renin levels  
2) CLIA vs. RIA of serum aldosterone levels  
3) CLIA vs. IRMA of renin levels between serum and plasma  
4) CLIA vs. RIA of aldosterone levels between serum and plasma  
5) Aldosterone (serum, plasma) to renin (plasma) ratios for CLIA and for RIA

**Statistical analysis**
The statistical analysis was conducted using MedCalc version 4.31.010. Continuous variables are expressed as means and 95% confidence interval (CI) for the mean. For continuous variables, Bland-Altman plots, Passing-Bablok regression analysis and Pearson’s correlation coefficient ($r_p$) were used to assess differences of data measured by CLIA and radioimmunoassay (RIA, IRMA). Preselected level of significance was $P<0.05$.

**Results**

**Comparison of EDTA plasma renin levels between CLIA and IRMA**
For the evaluation of plasmatic renin levels differences between CLIA and IRMA 79 samples were measured. Statistical characteristics of renin concentrations using CLIA and IRMA are reported in Table 1. Pearson’s correlation coefficient is: $r_p=0.965$ (slope = 0.964, intercept = –0.654, $P=0.062$) demonstrates sufficient compliance between CLIA and IRMA.

**Comparison of serum aldosterone levels between CLIA and RIA**
For the evaluation of serum aldosterone level differences between CLIA and RIA 90 samples were measured. Table 1 contains statistical parameters. Serum aldosterone levels using CLIA are lower in comparison with serum aldosterone levels using RIA. Pearson’s correlation coefficient is: $r_p=0.933$ (slope = 0.607, intercept = 23.81, $P<0.001$). Differences in average values, medians and standard deviations are reported in Table 1.

**Comparison of aldosterone levels obtained by RIA and CLIA for serum and EDTA plasma**
We used RIA to compare aldosterone concentrations (serum vs. plasma, $n=45$) and CLIA to compare aldosterone concentrations (serum vs. plasma, $n=43$). Basic statistical characteristics is shown in Table 2 (RIA, CLIA). The results of the Bland-
### Table 1 – Statistical characteristics of measuring serum aldosterone by CLIA vs. RIA and of measuring plasma renin by CLIA vs. IRMA

| Statistical parameters | Aldosterone serum levels | Renin plasma levels |
|------------------------|-------------------------|---------------------|
|                        | CLIA        | RIA       | CLIA       | IRMA       |
| Sample size            | 90          | 90        | 79         | 79         |
| Range (ng/l)           | 24.0–536.0  | 26.4–840.2| 2.4–121.58 | 1.7–140.3  |
| Arithmetic mean (ng/l) | 153.41      | 195.31    | 29.35      | 27.71      |
| 95% CI for the mean    | 132.32–174.50| 161.07–229.56| 23.53–35.17| 21.30–34.12|
| Median (ng/l)          | 124.50      | 159.70    | 22.09      | 18.90      |
| Pearson’s correlation coefficient ($r_p$) | 0.933 (0.899–0.955) | 0.965 (0.945–0.977) |
| Slope                  | 0.607 (0.610–0.750) | 0.964 (0.898–1.049) |
| Intercept              | 23.81 (13.83–31.32) | –0.654 (–1.687–[–0.007]) |
| Paired samples t-test (T) | 5.066      | 1.892     |            |            |
| Degrees of freedom (DF) | 89         | 78        |            |            |
| $P(T\leq t)$ two-tail  | <0.001      | 0.062     |            |            |
| t (t critical two-tail)| 1.986       | 1.989     |            |            |

**CL** – confidence interval

### Table 2 – Statistical characteristics of measuring aldosterone levels by RIA and by CLIA between serum and EDTA plasma

| Statistical parameters | Aldosterone levels by RIA | Aldosterone levels by CLIA |
|------------------------|---------------------------|---------------------------|
|                        | serum | EDTA plasma | serum | EDTA plasma |
| Sample size            | 45    | 45          | 43    | 43          |
| Range (ng/l)           | 6.6–359.3 | 8.1–391.6 | 30.0–523.0 | 30.0–561.0 |
| Arithmetic mean (ng/l) | 111.96 | 134.01      | 131.32 | 131.32      |
| 95% CI for the mean    | 83.23–140.69 | 102.17–165.87 | 95.98–166.67 | 96.06–166.57 |
| Median (ng/l)          | 96.90 | 128.0       | 81.20  | 76.30       |
| Pearson’s correlation coefficient ($r_p$) | 0.980 (0.963–0.989) | 0.994 (0.988–0.997) |
| Slope                  | 1.137 (1.059–1.264) | 0.978 (0.938–1.016) |
| Intercept              | 5.35 (0.406–10.927) | 0.727 (–1.613–4.585) |
| Paired samples t-test (T) | –6.501    | 0.002      |            |            |
| Degrees of freedom (DF) | 44       | 42         |            |            |
| $P(T\leq t)$ two-tail  | <0.001    | 0.353      |            |            |
| t (t critical two-tail)| 2.015     | 2.017      |            |            |

**CL** – confidence interval

Comparison of Aldosterone and Renin Immunoassays
Figure 1 – The Bland-Altman plots and the Passing-Bablok regression line. A) The Bland-Altman plots for data of aldosterone between average levels of serum and plasma vs. ratio aldosterone levels of serum to plasma using RIA. With the representation of the limits of agreement (dot-and-dash line), from −1.96 SD to +1.96 SD (SD – standard deviation). B) The Passing-Bablok regression line of aldosterone concentrations between serum and plasma using RIA (Pearson’s r=0.980, P-value < 0.001, y = 1.137x + 5.35).
Comparison of Aldosterone and Renin Immunoassays

Figure 2 – The Bland-Altman plots and the Passing-Bablok regression line. A) The Bland-Altman plots for data of aldosterone between average levels of serum and plasma vs. ratio aldosterone levels of serum to plasma using CLIA. With the representation of the limits of agreement (dot-and-dash line), from −1.96 SD to +1.96 SD (SD – standard deviation). B) The Passing-Bablok regression line of aldosterone concentrations between serum and plasma using CLIA (Pearson’s r=0.994, P-value < 0.353, y = 0.978x + 0.727).
Altman analysis are shown on Figure 1A (RIA); Figure 2A (CLIA) and Passing-Bablog analysis on Figure 1B (RIA); Figure 2B (CLIA) respectively.

Average plasma aldosterone values determined by RIA are higher (19.7%) in comparison with serum values. Pearson’s regression analysis demonstrates correlation coefficient, $r_P=0.980$ (slope = 1.137, intercept = 5.35, P<0.001).

Average plasma aldosterone values determined using CLIA were not different in comparison with serum values. Pearson’s regression analysis demonstrates an acceptable correlation coefficient, $r_P=0.994$. The slope value of 0.978 and the intercept value 0.727 are also acceptable.

Comparison of renin levels with IRMA and CLIA between serum and EDTA plasma

To compare renin levels, we used CLIA technology (serum vs. plasma, n=38) and IRMA technology (serum vs. plasma, n=44). Table 3 contains basic statistical characteristics for IRMA and CLIA. The results of the Bland-Altman analysis are shown of Figure 3A (IRMA) and Figure 4A (CLIA) and Passing-Bablog analysis on Figure 3B (IRMA) and Figure 4B (CLIA).

Plasma renin levels determined using IRMA are lower by 34.4% on average in comparison to serum levels. The value of Pearson’s correlation coefficient is: $r_P=0.948$, as well as the slope value of 0.625 are acceptable, however, the intercept value of 0.126 is not sufficient.

Plasma renin levels determined using CLIA are higher by 46.3% on average in comparison to serum levels. The value of Pearson’s correlation coefficient is: $r_P=0.921$ (slope = 1.597, intercept = 0.332, P=0.011).

Comparison of aldosterone to renin ratios

We compared aldosterone to renin ratios (ng/dl) for CLIA and for RIA or IRMA: serum aldosterone to EDTA plasma renin and EDTA plasma aldosterone to EDTA plasma renin. Statistical characteristics of these ratios of aldosterone and renin are shown in Table 4 for CLIA and for RIA. For CLIA ratio values for aldosterone (serum or EDTA plasma) to EDTA plasma renin Pearson’s correlation coefficient is: $r_P=0.999$, slope 0.959 and intercept 0.014 is acceptable. For radioimmunoassay ratio values for aldosterone (serum or EDTA plasma) to EDTA renin ratios Pearson’s correlation coefficient is: $r_P=0.992$, slope 1.133 and intercept 0.059 is also acceptable.

Discussion

Contemporary laboratory diagnostics of primary hyperaldosteronism (PH) provides for the determination of aldosterone and renin analytical procedures that are easy to perform and more time affordable than nowadays used radioimmunoassay procedures. These are mainly automated non-isotopic determinations of aldosterone and renin, from which we tested CLIA. It seems that for the diagnostics of PH are preferred non-isotopic direct determinations of both aldosterone and renin and their
### Table 3 – Statistical characteristics of measuring renin levels by CLIA and IRMA between serum and EDTA plasma

| Statistical parameters | Renin levels by IRMA | Renin levels by CLIA |
|------------------------|----------------------|----------------------|
|                        | serum                | EDTA plasma          | serum                | EDTA plasma          |
| Sample size            | 44                   | 44                   | 38                   | 38                   |
| Range (ng/l)           | 1.10–148.5           | 0.60–127.2           | 0.60–267.9           | 0.60–309.0           |
| Arithmetic mean (ng/l) | 27.89                | 18.29                | 27.95                | 40.90                |
| 95% CI for the mean    | 17.83–37.95          | 10.78–25.80          | 9.66–46.25           | 17.40–64.41          |
| Median (ng/l)          | 14.60                | 9.50                 | 7.00                 | 10.85                |
| Pearson’s correlation coefficient (r<sub>p</sub>) | 0.948 (0.906–0.872) | 0.921 (0.853–0.959) |
| Slope                  | 0.625 (0.532–0.811)  | 1.597 (1.214–2.123)  |
| Intercept              | 0.126 (−1.882–1.531) | 0.332 (−1.877–2.523) |
| Paired samples t-test (T) | 5.127               | −2.693               |
| Degrees of freedom (DF)| 43                   | 37                   |
| P(T≤t) two-tail         | <0.001               | 0.011                |
| t (t critical two-tail) | 2.018                | 2.025                |

CI – confidence interval

### Table 4 – Statistical characteristics of aldosterone (serum, EDTA plasma) to of renin (EDTA plasma) ratios for radioimmunoassay and for CLIA

| Statistical parameters | Aldosterone to renin ratios for radioimmunoassay | Aldosterone to renin ratios for CLIA |
|------------------------|-------------------------------------------------|------------------------------------|
|                        | ALDO<sub>S</sub>/REN<sub>P</sub> | ALDO<sub>P</sub>/REN<sub>P</sub> | ALDO<sub>S</sub>/REN<sub>P</sub> | ALDO<sub>P</sub>/REN<sub>P</sub> |
| Sample size            | 38                  | 38                  | 27                   | 27                   |
| Range (ng/dl)          | 0.07–17.0           | 0.09–23.0           | 0.035–34.40          | 0.033–32.20          |
| Arithmetic mean (ng/dl)| 2.138               | 2.618               | 3.594                | 3.491                |
| 95% CI for the mean    | 0.856–3.421         | 0.964–4.272         | 0.782–6.406          | 0.835–6.147          |
| Median (ng/dl)         | 0.73                | 0.85                | 0.59                 | 0.74                 |
| Pearson’s correlation coefficient (r<sub>p</sub>) | 0.992 (0.985–0.997) | 0.999 (0.998–1.000) |
| Slope                  | 1.113 (1.049–1.298) | 0.959 (0.924–1.007) |
| Intercept              | 0.059 (0.003–0.100) | 0.014 (−0.001–0.038) |
| Paired samples t-test (T) | 2.34                | −1.09               |
| Degrees of freedom (DF)| 37                   | 26                   |
| P(T≤t) two-tail         | 0.025               | 0.286               |
| t (t critical two-tail) | 2.026                | 2.056               |

ALDO<sub>S</sub> – aldosterone of serum; ALDO<sub>P</sub> – aldosterone of EDTA plasma; REN<sub>P</sub> – renin of EDTA plasma; CI – confidence interval
Figure 3 – The Bland-Altman plots and the Passing-Bablok regression line. A) The Bland-Altman plots for data of EDTA plasma renin between average levels of serum and plasma vs. ratio renin levels of serum to plasma using IRMA. With the representation of the limits of agreement (dot-and-dash line), from $-1.96$ SD to $+1.96$ SD (SD – standard deviation). B) The Passing-Bablok regression line of renin levels between serum and plasma using IRMA (Pearson’s $r=0.948$, P-value < 0.001, $y = 0.625x + 0.126$).
Figure 4 – The Bland-Altman plots and the Passing-Bablok regression line. A) The Bland-Altman plots for data of EDTA plasma renine between average levels of serum and plasma vs. ratio plasma renine levels of serum to plasma using CLIA. With the representation of the limits of agreement (dot-and-dash line), from $-1.96 \text{ SD}$ to $+1.96 \text{ SD}$ ($\text{SD}$ – standard deviation). B) The Passing-Bablok regression line of plasma renin between serum and plasma using CLIA (Pearson’s $r=0.921$, $P$-value $= 0.011$, $y = 1.597x + 0.332$).
ratio value as well (Dorrian et al., 2010; Horváth et al., 2012; Burrello et al., 2015; Glinicki et al., 2015).

PH laboratory diagnostics is based over last 45 years also on the ratio of aldosterone and renin or renin activity respectively (Ferrari et al., 2004). Its usage helps to find out patients with PH and distinguish between those who bear the disease and those who do not. Test of renin plasmatic activity is continuously replaced by concentration measurement only (Reincke et al., 2003; Ferrari et al., 2004). Optimization of the ratio for screening purposes is being studied (Jensen et al., 2014; Ma et al., 2018; Russmann et al., 2019). Till now none widely used optimized ratio calculation is known. It is probably caused by different analytical procedures used (RIA, CLIA, ELISA), by the choice of biological material (serum, plasma), by the usage of renin concentration or renin activity and by different units. Even more there is no international consensus on aldosterone/renin ratio cut-off and no guidelines for its interpretation. Discussion published in Clinical Chemistry shows non-comprehensive opinions on ratio usage in screening of PH (Raizman et al., 2015; Vecchiola et al., 2019).

Most studies on PH diagnostics are focused more on optimizing ratio of aldosterone to renin or renin activity calculation, but they do not address correlation between serum and plasmatic levels. In the study by Belaidi et al. (2015), who measured aldosterone in serum using RIA assay (coat-a-count, Siemens, Marburg, Germany) and CLIA using a LIAISON automated analyser (Diasorin, Saluggia, Italy) RIA and CLIA aldosterone serum concentration were linearly correlated with a slope of 0.988 and an intercept of 70.4 pmol/l. The variations of aldosterone serum concentration obtained with two assays during postural tests were very consistent. Contrary to it in our comparison of serum aldosterone levels between RIA (Beckman) and CLIA (DiaSorin) slope was 0.607 and intercept 23.81 pmol/l.

Glinicki et al. (2015) studied the comparison of aldosterone levels between the serum and EDTA plasma. Aldosterone was measured by RIA (ZenTech, RIAZENco, Belgium). Measured concentrations of aldosterone in plasma (EDTA2K) and serum samples showed high correlation ($r_P = 0.979$). The differences between pairs of plasma and serum samples ranged from 37% to 144% (median 75%) (Belaidi et al., 2015). In our study concentrations of aldosterone in serum and EDTA plasma were measured by RIA (Beckman) with correlation 0.980, while differences between pairs of plasma and serum sample were lower 5% to 85% (median 30.6%). Good regression dependency determination of aldosterone in serum between CLIA and RIA was shown. We found the significant effect of sample material (serum vs. EDTA plasma), except of aldosterone determination using CLIA ($r_P = 0.994$, slope = 0.978, intercept = 0.727).

Statistical evaluation shows that the choice of material for the determination of renin influences both IRMA and CLIA methods. Renin concentration measurements show significant variations between measurement serum and plasma however both systems have a similar range of the calibration curve (IRMA to 320 pg/ml, CLIA to
316.5 pg/ml) and in both assay systems two mouse monoclonal antibodies are used. The first monoclonal antibody is fixed to the solid phase anti-renin and prorenin (IRMA wall tubes, CLIA, magnetic particles). The second monoclonal antibody specific to renin is labelled with a corresponding detection substance (IRMA, $^{125}$I, CLIA, isoluminol). The assays are referenced to the World Health Organization International Reference Preparation, NIBSC code 68/356. Our values of primary concentration of renin show considerable variability between serum and plasma, which does not yet support determining renin in serum.

The discrepancies among renin assay results could be caused by different specificity of antisera or antibodies may vary between assays. Most commonly it is due to heterophile antibodies or due to endogenous circulating antibodies, of cross-reacting steroids or other interfering substances (Lonati et al., 2014).

Contemporary laboratory diagnostics of PH provides for the determination of aldosterone and renin analytical procedures that are easy to perform and time affordable than radioimmunoassay procedures. These are mainly the automated non-isotopic determination of aldosterone and renin, from which we tested CLIA (Dorrian et al., 2010; Jensen et al., 2014; Burrello et al., 2015).

The main advantage of CLIA is analysis quickness (renin 38 minutes, aldosterone 55 minutes) and shortening of the turnaround time. It is also the main reason for daily availability of determinations, for RIA methods the real frequency is usually just once or twice a week.

**Conclusion**

Current requirements on biochemistry laboratories include increasing workflow and productivity with rapid responses from the laboratory to clients. In contrast to radioimmunoassay methods, the automated non-isotopic technology improves analytical comfort and the possibility of sample processing completion on the day of receipt by the laboratory. Our study has shown that these requirements are met by a fully automated immunoassay LIAISON XL, which has enabled us fluently transfer the determination of aldosterone and renin from RIA/IRMA to CLIA method. We found the significant effect of sample material (serum vs. plasma) with exception of aldosterone determination using CLIA. We can conclude that our results show good concordance also in plasmatic but not in serum renin (IRMA vs. CLIA). Automated aldosterone and renin chemiluminescent assays are a reliable alternative to the radio-immunometric method. Aldosterone to renin ratios (ng/dl) for CLIA and for RIA or IRMA (both serum aldosterone to EDTA plasma renin and EDTA plasma aldosterone to EDTA plasma renin) are comparable and any of them can be used. The main advantage of CLIA methods are good standardization, automatization and simple sample processing.

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