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# Rapid screening of primary aldosteronism by a novel chemiluminescent immunoassay

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## Methods

### Diagnosis of primary aldosteronism (PA) and subtype differentiation

We measured both plasma aldosterone and renin activity by conventional radioimmunoassays under withdrawal of anti-hypertensive agents that interfere with renin-angiotensin-aldosterone system but in the presence of calcium channel blocker and/or alpha1 adrenergic antagonists prescribed to take adequate control of hypertension during the study period. Those patients who showed an aldosterone-over-renin activity ratio (ARR) equal to or higher than 20 ng/dL per ng/mL/h, at baseline and after a captopril challenge test, were diagnosed as PA.[1] Those who did not fulfil the diagnostic criterion described above were additionally screened for other secondary causes of hypertension, and finally diagnosed to have essential hypertension (EH). Patients who showed other causes of secondary hypertension, such as autonomous secretion of cortisol detected by overnight low dose (1mg) dexamethasone suppression test, paraganglioma, other endocrine causes, and renovascular diseases detected by ultrasonography and/or computed tomography scans, were excluded from the study. PA patients with a desire for surgery underwent adrenal venous sampling (AVS), and the patients diagnosed with unilateral hyperaldosteronism by AVS underwent laparoscopic adrenalectomy as previously reported.[1-3] Aldosterone-producing lesions in the resected adrenals were confirmed by pathological evaluation and immunohistochemistry of steroidogenic enzymes including CYP11B1/2, 3BHSD1/2, and CYP17A.[4,5]

### Conventional radioimmunoassay of plasma aldosterone and active renin concentrations, and renin activity

Plasma aldosterone was measured by RIA using the commercially available SPAC-S Aldosterone Kit (Fujirebio Inc., Tokyo, Japan) and PRA was measured by RIA for angiotensin I using the commercially available Renin RIABEAD Kit (Fujirebio Inc.). Plasma active renin was measured using the commercially available Renin IRMA Kit (Fujirebio Inc.). Using each commercially available assay listed above, the analytical sensitivity of plasma aldosterone, renin activity and active renin concentrations was 2.5 ng/dL, 0.1 ng/mL/h and 2.0 pg/mL, respectively. Plasma samples for aldosterone, renin activity and active renin concentration were obtained with the patient in a recumbent position early in the morning after 30 minutes of bed rest. Plasma ACTH was measured by electro-chemiluminescent immunoassay using the commercially available ECLusys ACTH kit (Roche Diagnostics K.K., Tokyo) and serum cortisol was measured by chemiluminescent immunoassay using the commercially available Chemilumi ACS-E Cortisol Kit (Siemens Healthcare Diagnostics, Inc., Tokyo).

## Description of the novel assay protocol

Accuraseed system is an automated immunoassay system for simultaneous measurement of plasma aldosterone concentration (PAC) and active renin concentration (ARC) (Wako Pure Chemical Industries, Ltd., Osaka, Japan). Accuraseed was developed using the newly developed magnetic particles called MAGRAPID (Japanese Unexamined Patent Application Publication No. 2016-105066, US 9,157,911 B2). Accuraseed, based on a chemiluminescent enzyme immunoassay, makes it possible to simultaneously measure PAC and ARC from a plasma sample, and the total assay time for both PAC and ARC is 10 minutes and 20 seconds. Two assays, one for PAC and the other for ARC, are performed in parallel from a single specimen. The required time is defined as follows; it starts when centrifuged plasma sample is applied and it ends when assay result is provided from Accuraseed system, not including time for sample processing before assay.

## aldosterone concentration

The aldosterone assay is designed as a competitive immunoassay format. For determination of PAC, we used a highly specific mouse anti-aldosterone monoclonal antibody (A2E11) [6] and peroxidase-conjugated aldosterone. The assay protocol was as follows; 25 µL of plasma were mixed with 50 µL of reagent #1 including mouse anti-aldosterone monoclonal antibody and goat anti-mouse polyclonal antibody, immobilized onto the magnetic particles, and incubated for 180 seconds at 37 °C. After incubation, 50 µL of reagent #2 including peroxidase-conjugated aldosterone were added and the mixture was incubated for 180 seconds at 37 °C. After removing the unbound conjugate solution and washing the magnetic particles for 60 seconds, 50 µL of reagent #3 including 8-amino-5-chloro-7-phenylpyrido[3,4]pyridazine-1,4-(2H,3H)dione sodium salt (L-012) as the highly sensitive luminescent reagent was mixed with 100 µL of reagent #4 including hydrogen peroxide, to measure chemiluminescence. The amount of peroxidase-conjugated aldosterone bound to the magnetic particles is inversely proportional to the PAC in the sample, and then aldosterone in the sample is calculated from the calibration curve prepared with standard solutions.

## active renin concentration

For determination of active renin concentration, we used two anti-renin monoclonal antibodies (Japanese patent No. 2877222); one binds to only renin (monoclonal antibody #11-6) and the other binds to renin and prorenin (monoclonal antibody #12-12). The assay protocol was as follows; 25 µL of plasma were mixed with 50 µL of reagent #1, including anti-renin monoclonal antibody (#12-12), which was immobilized onto the magnetic particles, and incubated for 180 seconds at 37 °C. After removing the mixed solution and washing the magnetic particles for 60 seconds, 50 µL of reagent #2, including peroxidase-conjugated anti-active renin monoclonal antibody (#11-6), was added and the mixture was

incubated for 180 seconds at 37 °C. After removing reagent #2 and washing the magnetic particles for 60 seconds, 100 µL of reagent #3, including L-012 as high sensitive luminescent reagent, were mixed with 100 µL of reagent #4 including hydrogen peroxide, to measure chemiluminescence. The amount of the peroxidase-conjugated antibody bound to the magnetic particles increases in proportion to the ARC in the sample; ARC in the sample is calculated from the calibration curve prepared by using standard solutions.

Effect of storage temperature on the ARC assay

Effect of storage temperature

Influence of storage temperature on stability of ARC measurement was analyzed as follows; 5 plasma samples were employed and each sample was aliquoted into 7 test tubes. One of the seven aliquots per each plasma sample was measured by the ARC assay immediately (0h), and other six aliquots were stored at the following temperatures, on ice (0℃), 5℃ or 26℃, and then applied to measurement after 1, 3, 6, 9, 12 and 24 hour of incubation. Each assay was performed in duplicate. The mean of measurements at each time point (1/3/6/9/12/24h) was compared to result of baseline sample (0h). Relative values were calculated as follows; % relative value = [each time point's mean observed value] / [baseline (0h) mean observed value].

Effect of freeze-thaw cycles

Influence of freeze-thaw cycles on the ARC measurement was analysed as follows; 3 plasma samples were employed and each sample was aliquoted into 4 test tubes. One of the four aliquots per each plasma sample was measured by the ARC assay immediately (0 cycle), and other three aliquots were stored -80℃, and then thawed using 20℃ water bath to room temperature (meaning 1 cycle), and repeated this freeze-thaw processes three (3 cycle) and five times (5 cycle) before application to the ARC assay. Each assay was performed in duplicate. The mean of measurements at each cycle (1/3/5 cycle) was compared to result of baseline sample (0 cycle). Relative values were calculated as follows; % relative value = [each cycle's mean observed value] / [baseline (0 cycle) mean observed value].

Measurement of renin standard for comparison to preceding ARC assays

After development of the ARC assay including calibration process using human recombinant renin (human activated renin (GenBank Accession No. NM_000537), amino acids 67-406 with C-terminal HIS tag, Catalog#: 80200, Lot#: 120228, BPS Bioscience Inc., San Diego, CA), we assayed international standard of renin (WHO International Standard renin, human, NIBSC code: 68/356) for comparison to preceding ARC assays.

## Bland-Altman plot analysis

Bland-Altman plot was performed to analyze agreement between two different assays and show a bias and limits of agreement with 95% confidence interval.

## Concept and design of Accuraseed system

Accuraseed system was developed for both clinic and hospital use. For screening purpose of PA in clinic settings, on-site and faster availability of assay results might be a help in making clinical decision whether further workup is indicated or not. Additionally, in the hospital settings, this faster assay system might shorten waiting time needed to obtain results of confirmation tests and, if indicated, subsequent adrenal venous sampling, and time-saving effects provided by this assay system might be accumulated throughout the whole diagnostic processes from screening to lateralization. The Accuraseed auto-analyzer system is originally designed to fit relatively small space even in clinic settings and ready to provide clinically useful assays other than those of aldosterone and renin, especially in the field of thyroid medicine, cardiology and oncology, considering versatility in hospital settings.

# Results

## Laboratory validation of the novel assays for PAC and ARC

For the PAC and ARC assays, the limits of detection were 5.0 ng/dL and 0.1 pg/mL, respectively, as shown in Tables S1 and S2. Limits of quantification at 20% and 15% coefficient of variation were found to be 5.0 ng/dL and 0.1 pg/mL, respectively (Figure S1 and S2). Similarly, analytical sensitivities were revealed to be 5.0 ng/dL and 0.1 pg/mL for the PAC and ARC assays, respectively. Assay ranges (defined as analytical sensitivity to upper limit of detection) were set to be 5.0-160.0 ng/dL and 0.1-500.0 pg/mL for PAC and ARC assays, respectively. The results of accuracy experiments are shown in Tables S3 and S4 for the PAC and ARC assays, respectively; those of intra- and inter-assay precision are shown in Tables S5 and S6 for PAC and ARC assays, respectively; and linearity of results obtained by PAC and ARC assays are shown in Tables S7 and S8, respectively. Detailed results of recovery, interference and cross-reactivity for PAC assay are shown in Tables S9, S10 and S11, respectively. Similarly, results of recovery and interference for the ARC assay are presented in Tables S12 and S13, respectively.

## Sensitivity testing

The results of LoD are shown in Tables S1 and S2, and the results of LoQ are shown in Figures S1and S2

aldosterone

LoD should be calculated by performing 21 measurements of Zero matrix and samples with very low concentrations (3 concentrations; samples may be diluted in a Zero matrix). LoD was the lowest concentration determined using the following condition: [Average-2SD (Zero matrix)] > [Average+2SD (low sample)]

Four low level samples should be obtained for the determination of LoQ. These may be created by dilution of samples in a Zero matrix. Samples should be assayed ten times. LoQ was determined as the concentration corresponding to the 20% CV cut-off.

Renin (ARC)

LoD should be calculated by performing 21 measurements of Zero matrix and samples with very low concentrations (5 concentration; samples may be diluted in a Zero matrix). LoD was the lowest concentration determined using the following condition: [Average+2SD(Zero matrix)] < [Average-2SD(low sample)]

Five low level samples should be obtained for the determination of LoQ. These may be created by dilution of samples in a Zero matrix. Samples should be assayed ten times. LoQ was determined as the concentration corresponding to the 15% CV cut-off.

Accuracy

The results of the accuracy are shown in Tables S3 and S4. Accuracy studies were performed using six samples (three plasma and three standards).

Precision

The results of the inter and intra-assay precision tests are shown in Tables S5 and S6.
Inter-assay precision studies were performed in 21repricate measurements using plasma from 3 patients.
Intra-precision studies were performed in duplicate using plasma from 2 patients (6 runs over 15days).

Linearity

The results of the linearity tests are shown in Tables S7 and S8. Two samples with a low and high concentration of aldosterone were assayed alongside the diluted samples using the aldosterone assays. Three samples with a low and high concentration of ARC were assayed alongside the diluted samples using the renin assays. Each dilution was tested in duplicate. The mean observed values were compared to the expected values. The OBS/EXP was calculated for the intermediate dilutions, to calculate the percent recovery, determined using the following formula:

% Recovery = [Mean observed value]/[ Expected value]  x 100

Recovery

The results of recovery tests are shown in Tables S9 and S12.

aldosterone

The study to assess spiking recovery by the aldosterone assay was performed by spiking 3 EDTA plasma samples with the reference standard. The spike concentrations were three, giving a mean increase in Aldosterone concentration of 50.0 to 400.0pg/mL, respectively in the three samples. The control (0% spike) and spiked samples were run in triplicate. The samples were spiked and placed directly on the analyser for measurement.

Recovery was calculated as follows:
% Recovery = [Observed Recovery value / Expected Recovery value (Analyte added)] x 100
The Accuraseed Aldosterone assay was designed to have a minimum acceptable recovery of 75-125 %.

renin (ARC)

The study to assess spiking recovery by the renin (ARC) assay was performed by spiking 3 EDTA plasma samples with the reference standard. The spike concentrations were three, giving a mean increase in renin concentration of 2.0 to 100.0pg/mL, respectively in the three samples. The control (0% spike) and spiked samples were run in triplicate. The samples were spiked and placed directly on the analyser for measurement.

Recovery was calculated as follows:
% Recovery = [Observed Recovery value / Expected Recovery value (Analyte added)] x100
The renin (ARC) assay was designed to have a minimum acceptable recovery of 80-120%.

Interference

The results of the interference are shown in Tables S10 and S13.

aldosterone

Interference testing was performed for the following substances: ascorbic acid, hemoglobin, bilirubin, bilirubin-conjugate, chyle and rheumatoid factor. To determine potential interference in the specific detection, two base plasma samples were spiked with the potential interferent at five concentrations. Control samples (blank) were spiked with a volume of relevant diluent equal to that of the spiked interferent. Spiked and control samples (blank) were then compared. The differences observed between the spiked and control sample values were examined and assessed according to acceptance criterion. The criterion for pass or fail of the assay as stated in the protocol was ≤25% concentration bias to the un-spiked sample.

Interference was calculated as follows:
% Interference = [Sample value (Interferent spiked) / Control Sample value (Blank)] x 100
The aldosterone assay was designed to have a minimum acceptable interference of 75-125 %.

renin (ARC)

Interference testing was performed as already described for aldosterone. The criterion for pass or fail of the assay as stated in the protocol was ≤20% concentration bias to the un-spiked sample.

Interference was calculated as:
% Interference = [Sample value (Interferent spiked) / Control Sample value (blank)] x 100
The rennin (ARC) assay was designed to have a minimum acceptable interference of 80-120 %.

Cross-reactivity

aldosterone

The results of the cross-reactivity test are shown in Table S11

Cross-reactivity was defined as the point where the reduction in signal corresponds to 50% of the signal achieved in the absence of the analyte (B/Bo of 50%), as a percentage of the analyte concentration giving the same fall in signal

% Cross-reactivity = [$ED_{50}$ (ng/dL) aldosterone]/[$ED_{50}$ (ng/dL) compound] x 100

Stock concentrations of the substances to be checked for cross-reactivity were prepared initially in an organic solvent. The stock solution of the cross-reactant was then spiked into the standard solution and subsequently diluted down serially to create a 5-point standard curve for each substance. Curves were run in the same experiment for comparison of $ED_{50}$ values of the potential cross-reactant against the $ED_{50}$ of the aldosterone assay displacement curve.

Effect of storage temperature and freeze-thaw cycles on ARC assay

The results of storage temperature and freeze-thaw cycles are shown in Table S14 through S17 and Figure S3 through S6.

Measurement of renin standard for comparison to preceding ARC assays

The ARC assay showed that 1 international unit (IU) of WHO renin standard was equal to 591 ng. Based on the measurement of WHO standard of renin, we also obtained unit conversion factor of 1.692 from pg/mL to μIU/mL.

Bland-Altman plot analysis

Bland-Altman plot analysis of PAC revealed a bias of -19.7 and the limits of agreement were -2.18 and -37.16 with 95% confidence interval when comparing the novel CLEIA and the conventional RIA (Figure S2A).

Bland-Altman plot analysis revealed a bias of 13.7, and the limits of agreement were 10.85 and 16.55 with 95% confidence interval when CLEIA and LC-MS/MS of PAC were compared (Figure S2B).

Bland-Altman plot analysis between RIA and LC-MS/MS of PAC revealed a bias of 33.4 with the limits of agreement of 15.23 and 51.51 with 95% confidence interval (Figure S2C).

Bland-Altman plot analysis of ARC revealed the bias of -0.97 and the limits of agreement were -1.087 and -0.8671 with 95% confidence interval when comparing CLEIA and the conventional RIA (Figure S2D).

Estimation of clinical benefit and cost in Accuraseed system

In our practice in Sendai, all assays using radioactive materials are performed in referral laboratory centers, away from our hospital. Thus, the waiting time for radioimmunoassay results ranges from three to five business days. While we assume that a total of four to five occasions of the measurement is necessary, i.e., one or two occasions in screening, two occasions in two confirmation tests, and one occasion in AVS, we could save at least 12-15 days over the whole course of workup by introducing the rapid assay system.

Typical clinical scenario is expected as follows; a patient is referred to our institution with a positive screening test and blood chemistry results including electrolytes and renal function tests. Subsequently, at first visit, physicians make plan for confirmatory tests, usually two tests. While there used to be waiting time, 3-5 days per test, for results to come back when radioimmunoassays were employed, results of the faster assay system are available within the

same day, meaning immediate decision might be made whether next lateralization step is indicated or not. When adrenal venous sampling is performed, diagnosis on lateralization and subsequent indication for adrenalectomy can be made within the same day, also suggesting substantially shorter time from first visit to the final clinical decision.

Moreover, in settings of inpatient care, similar effects might be prominent in terms of reduction in the number of clinic visits and time-saving. When a patient with a positive screening test is referred and admitted to our center, two confirmatory tests and adrenal venous sampling could be performed in consecutive three days, because the faster assay system made it possible to decide immediately whether a next step or surgery is indicated or not within the day, suggesting duration of hospital stay for whole workup for PA might be substantially reduced, compared to era dependent on radioimmunoassays.

Finally, accumulated effects provided by shortening of the workup time in each case are expected to improve throughput of potential patients who are positive for screening and waiting for workup in PA centers, and synergistically increase the chance to diagnose patients who were otherwise undiagnosed and/or medically treated despite uncovered indication for surgical cure.

As far as cost is concerned, initial cost needed to introduce the new system was estimated to be approximately 16 million yen and running cost per test was decided to be approximately 1300 and 1100 yen (measurements of PAC and ARC, respectively) in Japan's national healthcare service system. Assay costs to be paid by patient makes little difference between conventional radioimmunoassay and the new enzyme assay in Japan's national healthcare service system, meaning no additional cost per patient with introduction of the faster assay system.

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**Table S1.** Limit of detection in CLEIA of PAC.

(CPS)

| sample | 0 pg/mL | 50 pg/mL | 75 pg/mL | 100 pg/mL |
|---|---|---|---|---|
| 1 | 556278 | 522638 | 481819 | 486993 |
| 2 | 570413 | 509682 | 505019 | 475918 |
| 3 | 581653 | 508379 | 495884 | 487489 |
| 4 | 565041 | 518878 | 488245 | 453194 |
| 5 | 559428 | 520852 | 505633 | 476671 |
| 6 | 568207 | 507232 | 487754 | 482728 |
| 7 | 557074 | 521782 | 504025 | 478575 |
| 8 | 553342 | 515424 | 483717 | 484343 |
| 9 | 560665 | 524126 | 479336 | 495881 |
| 10 | 552065 | 526536 | 507562 | 505416 |
| 11 | 548083 | 522161 | 484546 | 477261 |
| 12 | 548989 | 500419 | 506978 | 481149 |
| 13 | 571740 | 534819 | 497657 | 472539 |
| 14 | 556007 | 518977 | 513987 | 487883 |
| 15 | 545188 | 530980 | 516795 | 483351 |
| 16 | 544314 | 509890 | 489858 | 458871 |
| 17 | 549769 | 524062 | 507235 | 500366 |
| 18 | 557958 | 513750 | 499361 | 501496 |
| 19 | 549778 | 522943 | 506527 | 487342 |
| 20 | 565754 | 497098 | 505806 | 480633 |
| 21 | 565385 | 520166 | 511517 | 483507 |
| n | 21 | 21 | 21 | 21 |
| average | 558435 | 517657 | 499012 | 482934 |
| S.D. | 9765 | 9493 | 11342 | 12500 |
| C.V. | 1.7% | 1.8% | 2.3% | 2.6% |
| average+2S.D. | 577965 | 536643 | 521696 | 507934 |
| average-2S.D. | 538905 | 498671 | 476328 | 457934 |

CLEIA indicates chemiluminescent enzyme immunoassay; PAC, plasma aldosterone concentration.

**Table S2.** Limit of detection in CLEIA of ARC.

(CPS)

| sample | 0.0 pg/mL | 0.1 pg/mL | 0.2 pg/mL | 0.3 pg/mL | 0.5 pg/mL | 0.7 pg/mL |
|---|---|---|---|---|---|---|
| 1 | 1716 | 3249 | 5161 | 6392 | 9825 | 13517 |
| 2 | 1816 | 3321 | 4820 | 6336 | 9909 | 13722 |
| 3 | 1833 | 3207 | 5114 | 6242 | 9656 | 13521 |
| 4 | 1692 | 3390 | 4928 | 6207 | 9618 | 13320 |
| 5 | 1680 | 3266 | 4817 | 6265 | 9406 | 13593 |
| 6 | 1665 | 3279 | 4842 | 6349 | 9829 | 13530 |
| 7 | 1655 | 3219 | 4762 | 6211 | 9697 | 13200 |
| 8 | 1759 | 3357 | 4932 | 6340 | 9565 | 13124 |
| 9 | 1713 | 3210 | 5050 | 5984 | 9514 | 13317 |
| 10 | 1638 | 3272 | 4661 | 6118 | 9575 | 13163 |
| 11 | 1642 | 3343 | 4885 | 6489 | 9765 | 12866 |
| 12 | 1715 | 3245 | 5163 | 6534 | 10227 | 13317 |
| 13 | 1668 | 3455 | 5099 | 6289 | 9863 | 13609 |
| 14 | 1752 | 3240 | 4953 | 6660 | 9876 | 13155 |
| 15 | 1720 | 3310 | 4781 | 6383 | 9784 | 13419 |
| 16 | 1732 | 3226 | 4954 | 6588 | 9649 | 13394 |
| 17 | 1540 | 3256 | 4653 | 6190 | 10049 | 13208 |
| 18 | 1732 | 3182 | 4815 | 6679 | 10100 | 13008 |
| 19 | 1719 | 3197 | 4815 | 6229 | 9924 | 13665 |
| 20 | 1697 | 3662 | 4769 | 6504 | 9823 | 13358 |
| 21 | 1545 | 3233 | 4643 | 6255 | 9549 | 13302 |
| n | 21 | 21 | 21 | 21 | 21 | 21 |
| average | 1697 | 3291 | 4887 | 6345 | 9772 | 13348 |
| S.D. | 71.5 | 109.2 | 160.4 | 178.9 | 206.3 | 221.7 |
| C.V. | 4.2% | 3.3% | 3.3% | 2.8% | 2.1% | 1.7% |
| average+2S.D. | 1840 | 3510 | 5207 | 6703 | 10184 | 13791 |
| average-2S.D. | 1554 | 3073 | 4566 | 5987 | 9359 | 12905 |

CLEIA indicates chemiluminescent enzyme immunoassay; ARC, active renin concentration.

**Table S3.** Accuracy in CLEIA of PAC.

| analyte | plasma | | standard | |
|---|---|---|---|---|
| sample | aldosterone (pg/mL) | accuracy (%) | aldosterone (pg/mL) | accuracy (%) |
| 1 | 119.2 | 112.3% | 139.8 | 97.8% |
| | 96.8 | 91.2% | 139.0 | 97.2% |
| | 110.1 | 103.8% | 139.7 | 97.7% |
| 2 | 418.5 | 96.2% | 477.9 | 96.4% |
| | 385.4 | 88.6% | 490.5 | 99.0% |
| | 399.5 | 91.8% | 464.2 | 93.7% |
| 3 | 1067.4 | 101.8% | 1371.2 | 100.8% |
| | 1051.9 | 100.3% | 1406.6 | 103.4% |
| | 1071.1 | 102.1% | 1368.3 | 100.6% |

CLEIA indicates chemiluminescent enzyme immunoassay; PAC, plasma aldosterone concentration.

**Table S4.** Accuracy in CLEIA of ARC.

| analyte | plasma | | standard | |
|---|---|---|---|---|
| sample | ARC (pg/mL) | accuracy (%) | ARC (pg/mL) | accuracy (%) |
| 1 | 6.6 | 104.8% | 5.8 | 96.7% |
| | 6.5 | 103.2% | 5.7 | 95.0% |
| | 6.6 | 104.8% | 6.0 | 100.0% |
| 2 | 58.3 | 98.8% | 60.1 | 100.2% |
| | 59.1 | 100.2% | 60.2 | 100.3% |
| | 60.4 | 102.4% | 59.5 | 99.2% |
| 3 | 371.5 | 100.4% | 410.6 | 102.7% |
| | 370.6 | 100.2% | 412.4 | 103.1% |
| | 371.6 | 100.4% | 408.1 | 102.0% |

CLEIA indicates chemiluminescent enzyme immunoassay; ARC, active renin concentration.

**Table S5.** Inter, intra-assay precision in CLEIA of PAC.

Inter-assay precision

(pg/mL)

| sample | plasma 1 | plasma 2 | plasma 3 |
|---|---|---|---|
| n | 21 | 21 | 21 |
| average | 181.8 | 457.3 | 1133.5 |
| range | 44.3 | 44.7 | 64.9 |
| S.D. | 10.53 | 12.15 | 16.36 |
| C.V. | 5.8% | 2.7% | 1.4% |

Intra-assay precision

(pg/mL)

| time to assay | 0 days | 2 days | 7 days | 10 days | 13 days | 15 days | mean | Range | SD | CV |
|---|---|---|---|---|---|---|---|---|---|---|
| sample 1 | 153.0 | 194.8 | 162.1 | 179.7 | 153.0 | 163.4 | 167.7 | 41.8 | 16.49 | 9.8% |
| sample 2 | 457.0 | 464.1 | 473.6 | 482.0 | 457.0 | 467.8 | 466.9 | 25.0 | 9.77 | 2.1% |

CLEIA indicates chemiluminescent enzyme immunoassay; PAC, plasma aldosterone concentration.

**Table S6.** Inter-, intra-assay precision in CLEIA of ARC.

Inter-assay precision

(pg/mL)

| sample | plasma 1 | plasma 2 | plasma 3 |
|---|---|---|---|
| n | 21 | 21 | 21 |
| average | 3.8 | 48.9 | 360.0 |
| range | 0.3 | 4.8 | 20.4 |
| S.D. | 0.09 | 1.10 | 5.47 |
| C.V. | 2.4% | 2.2% | 1.5% |

Intra-assay precision

(pg/mL)

| time to assay | 0 days | 2 days | 7 days | 10 days | 13 days | 15 days | mean | Range | SD | CV |
|---|---|---|---|---|---|---|---|---|---|---|
| sample 1 | 3.1 | 2.9 | 3 | 3.2 | 3.1 | 3.1 | 3.1 | 0.3 | 0.10 | 3.2% |
| sample 2 | 44.1 | 43.9 | 43.3 | 44.6 | 44.2 | 45.2 | 44.2 | 1.9 | 0.64 | 1.4% |

CLEIA indicates chemiluminescent enzyme immunoassay; ARC, active renin concentration.

**Table S7.** Linearity in CLEIA of PAC.

| parameters | | sample 1 | sample 2 |
|---|---|---|---|
| aldosterone (pg/mL) | 0/10 | 0.0 | 0.0 |
| | 1/10 | 57.9 | 130.2 |
| | 2/10 | 116.3 | 256.2 |
| | 3/10 | 172.4 | 372.1 |
| | 4/10 | 216.6 | 515.8 |
| | 5/10 | 299.9 | 622.6 |
| | 6/10 | 345.4 | 732.7 |
| | 7/10 | 401.6 | 867.5 |
| | 8/10 | 451.1 | 998.0 |
| | 9/10 | 481.1 | 1110.7 |
| | 10/10 | 541.0 | 1214.2 |
| %observed /expected | 1/10 | 107% | 107% |
| | 2/10 | 107% | 106% |
| | 3/10 | 106% | 102% |
| | 4/10 | 100% | 106% |
| | 5/10 | 111% | 103% |
| | 6/10 | 106% | 101% |
| | 7/10 | 106% | 102% |
| | 8/10 | 104% | 103% |
| | 9/10 | 99% | 102% |
| | 10/10 | 100% | 100% |

CLEIA indicates chemiluminescent enzyme immunoassay; PAC, plasma aldosterone concentration.

**Table S8.** Linearity in CLEIA of ARC.

| parameters | | sample 1 | sample 2 | sample 3 |
|---|---|---|---|---|
| ARC (pg/mL) | 0/10 | 0.0 | 0.0 | 0.0 |
| | 1/10 | 3.8 | 15.5 | 46.4 |
| | 2/10 | 7.5 | 33.2 | 91.0 |
| | 3/10 | 11.6 | 48.4 | 141.3 |
| | 4/10 | 15.2 | 66.1 | 191.3 |
| | 5/10 | 18.5 | 78.5 | 242.5 |
| | 6/10 | 21.9 | 95.4 | 293.6 |
| | 7/10 | 25.4 | 108.9 | 331.1 |
| | 8/10 | 29.4 | 126.4 | 373.0 |
| | 9/10 | 32.9 | 145.2 | 416.0 |
| | 10/10 | 35.7 | 157.3 | 469.7 |
| %observed /expected | 1/10 | 106% | 99% | 99% |
| | 2/10 | 105% | 106% | 97% |
| | 3/10 | 108% | 103% | 100% |
| | 4/10 | 106% | 105% | 102% |
| | 5/10 | 104% | 100% | 103% |
| | 6/10 | 102% | 101% | 104% |
| | 7/10 | 102% | 99% | 101% |
| | 8/10 | 103% | 100% | 99% |
| | 9/10 | 102% | 103% | 98% |
| | 10/10 | 100% | 100% | 100% |

CLEIA indicates chemiluminescent enzyme immunoassay; ARC, active renin concentration.

**Table S9.** Recovery in CLEIA of PAC.

| sample | zero-spike (pg/mL) | expected -recovery (pg/mL) | spike (pg/mL) | recovery (pg/mL) | %recovery (%) |
|---|---|---|---|---|---|
| 1 | 161.1 | 50.0 | 214.4 | 53.3 | 106.6 % |
| 2 | 255.3 | 200.0 | 432.8 | 177.5 | 88.8 % |
| 3 | 750.9 | 400.0 | 1138.5 | 387.6 | 96.9 % |

CLEIA indicates chemiluminescent enzyme immunoassay; PAC, plasma aldosterone concentration.

**Table S10.** Interference in CLEIA of PAC

| interferent | concentration tested | PAC (pg/mL) | interference | PAC (pg/mL) | interference |
|---|---|---|---|---|---|
| ascorbic acid | 0 mg/dL | 120.0 | 100 % | 698.2 | 100 % |
| | 10 mg/dL | 132.2 | 110 % | 709.0 | 102 % |
| | 20 mg/dL | 131.3 | 109 % | 721.0 | 103 % |
| | 30 mg/dL | 131.2 | 109 % | 706.0 | 101 % |
| | 40 mg/dL | 131.6 | 110 % | 739.6 | 106 % |
| | 50 mg/dL | 137.5 | 115 % | 709.5 | 102 % |
| hemoglobin | 0 mg/dL | 161.6 | 100 % | 706.0 | 100 % |
| | 100 mg/dL | 149.5 | 93 % | 735.8 | 104 % |
| | 200 mg/dL | 143.9 | 89 % | 708.1 | 100 % |
| | 300 mg/dL | 162.3 | 100 % | 736.0 | 104 % |
| | 400 mg/dL | 161.4 | 100 % | 721.9 | 102 % |
| | 500 mg/dL | 153.4 | 95 % | 700.6 | 99 % |
| bilirubin | 0 mg/dL | 141.7 | 100 % | 712.9 | 100 % |
| | 3.8 mg/dL | 159.3 | 112 % | 713.2 | 100 % |
| | 7.6 mg/dL | 159.3 | 112 % | 706.8 | 99 % |
| | 11.3 mg/dL | 133.0 | 94 % | 711.4 | 100 % |
| | 15.1 mg/dL | 160.3 | 113 % | 710.6 | 100 % |
| | 18.9 mg/dL | 150.5 | 106 % | 701.7 | 98 % |
| bilirubin -conjugate | 0 mg/dL | 133.7 | 100 % | 687.4 | 100 % |
| | 4.2 mg/dL | 152.0 | 114 % | 665.9 | 97 % |
| | 8.3 mg/dL | 133.0 | 99 % | 693.8 | 101 % |
| | 12.5 mg/dL | 140.5 | 105 % | 698.1 | 102 % |
| | 16.7 mg/dL | 138.5 | 104 % | 671.9 | 98 % |
| | 20.8 mg/dL | 142.4 | 107 % | 693.4 | 101 % |
| chyle | 0 FTU | 135.4 | 100 % | 693.6 | 100 % |
| | 282 FTU | 138.2 | 102 % | 700.6 | 101 % |
| | 564 FTU | 132.9 | 98 % | 680.4 | 98 % |
| | 846 FTU | 133.9 | 99 % | 671.9 | 97 % |
| | 1,128 FTU | 121.9 | 90 % | 676.0 | 97 % |
| | 1,410 FTU | 118.8 | 88 % | 676.8 | 98 % |
| rheumatoid factor | 0 IU/mL | 138.4 | 100 % | 678.1 | 100 % |
| | 100 IU/mL | 140.7 | 102 % | 653.3 | 96 % |
| | 200 IU/mL | 159.6 | 115 % | 700.2 | 103 % |
| | 300 IU/mL | 165.2 | 119 % | 675.4 | 100 % |

CLEIA indicates chemiluminescent enzyme immunoassay; PAC, plasma aldosterone concentration.

**Table S11.** Cross-reactivity in CLEIA of PAC

| cross-reactants | cross-reactivity |
|---|---|
| Corticosterone | 0.00531 % |
| 18-hydroxycorticosterone | 0.11028 % |
| Cortisol | < 0.00015 % |
| Cortisone | < 0.00015 % |
| Deoxycorticosterone | 0.00065 % |
| Progesterone | < 0.00015 % |
| Tetrahydrocorticosterone | < 0.00015 % |
| Dexamethasone | < 0.00002 % |
| Prednisolone | 0.00002 % |
| Spironolactone | < 0.00015 % |
| Eplerenone | < 0.00015 % |

CLEIA indicates chemiluminescent enzyme immunoassay; PAC, plasma aldosterone concentration.

**Table S12.** Recovery in CLEIA of ARC

| sample | zero-spike (pg/mL) | expected -spike (pg/mL) | spike (pg/mL) | recovery (pg/mL) | %recovery (%) |
|---|---|---|---|---|---|
| 1 | 5.4 | 2.0 | 7.4 | 2.0 | 100.0 % |
| 2 | 40.2 | 20.0 | 59.2 | 19.0 | 95.0 % |
| 3 | 285.9 | 100.0 | 386.1 | 100.2 | 100.2 % |

CLEIA indicates chemiluminescent enzyme immunoassay; ARC, active renin concentration.

421 **Table S13.** Interference in CLEIA of ARC

| interferent | concentration | ARC (pg/mL) | interference | ARC (pg/mL) | interference |
|---|---|---|---|---|---|
| Ascorbic acid | 0 mg/dL | 35.8 | 100 % | 252.6 | 100 % |
| | 10 mg/dL | 36.1 | 101 % | 256.4 | 102 % |
| | 20 mg/dL | 36.4 | 102 % | 252.8 | 100 % |
| | 30 mg/dL | 36.2 | 101 % | 258.8 | 102 % |
| | 40 mg/dL | 36.3 | 101 % | 262.6 | 104 % |
| | 50 mg/dL | 37.2 | 104 % | 267.6 | 106 % |
| Hemoglobin | 0 mg/dL | 36.6 | 100 % | 268.4 | 100 % |
| | 100 mg/dL | 36.7 | 100 % | 264.3 | 98 % |
| | 200 mg/dL | 36.7 | 100 % | 269.2 | 100 % |
| | 300 mg/dL | 36.0 | 98 % | 264.5 | 99 % |
| | 400 mg/dL | 36.2 | 99 % | 270.8 | 101 % |
| | 500 mg/dL | 36.5 | 100 % | 271.4 | 101 % |
| Bilirubin | 0 mg/dL | 36.4 | 100 % | 272.5 | 100 % |
| | 3.8 mg/dL | 37.0 | 102 % | 272.8 | 100 % |
| | 7.6 mg/dL | 36.4 | 100 % | 269.5 | 99 % |
| | 11.3 mg/dL | 37.1 | 102 % | 272.4 | 100 % |
| | 15.1 mg/dL | 36.4 | 100 % | 272.0 | 100 % |
| | 18.9 mg/dL | 36.0 | 99 % | 265.3 | 97 % |
| Bilirubin -conjugate | 0 mg/dL | 37.7 | 100 % | 272.6 | 100 % |
| | 4.2 mg/dL | 36.7 | 97 % | 268.5 | 98 % |
| | 8.3 mg/dL | 37.4 | 99 % | 271.6 | 100 % |
| | 12.5 mg/dL | 38.5 | 102 % | 264.5 | 97 % |
| | 16.7 mg/dL | 36.8 | 98 % | 261.0 | 96 % |
| | 20.8 mg/dL | 37.5 | 99 % | 259.4 | 95 % |
| Chyle | 0 FTU | 37.4 | 100 % | 273.5 | 100 % |
| | 282 FTU | 37.1 | 99 % | 258.3 | 94 % |
| | 564 FTU | 36.5 | 98 % | 272.1 | 99 % |
| | 846 FTU | 36.3 | 97 % | 273.4 | 100 % |
| | 1,128 FTU | 36.8 | 98 % | 268.9 | 98 % |
| | 1,410 FTU | 34.1 | 91 % | 270.4 | 99 % |
| Rheumatoid factor | 0 IU/mL | 39.9 | 100.0 % | 283.5 | 100.0 % |
| | 110 IU/mL | 41.2 | 103.3 % | 281.3 | 99.2 % |
| | 220 IU/mL | 38.7 | 97.0 % | 279.1 | 98.4 % |
| | 330 IU/mL | 38.7 | 97.0 % | 286.2 | 101.0 % |

422 CLEIA indicates chemiluminescent enzyme immunoassay; ARC, active renin concentration.

**Table S14.** Cryoactivation of active renin concentration at 0°C

| storage time (hour) | relative value (v.s. 0 hour) | | | | |
|---|---|---|---|---|---|
| | plasma 1 | plasma 2 | plasma 3 | plasma 4 | plasma 5 |
| 0 | 100% | 100% | 100% | 100% | 100% |
| 1 | 105% | 106% | 114% | 110% | 111% |
| 3 | 110% | 123% | 119% | 113% | 134% |
| 6 | 116% | 118% | 125% | 114% | 136% |
| 9 | 112% | 125% | 129% | 116% | 136% |
| 12 | 121% | 134% | 135% | 122% | 148% |
| 24 | 132% | 158% | 149% | 130% | 152% |

**Table S15.** Cryoactivation of active renin concentration at 5°C

| storage time (hour) | relative value (v.s. 0 hour) | | | | |
|---|---|---|---|---|---|
| | plasma 6 | plasma 7 | plasma 8 | plasma 9 | plasma 10 |
| 0 | 100% | 100% | 100% | 100% | 100% |
| 1 | 101% | 100% | 106% | 100% | 104% |
| 3 | 102% | 93% | 109% | 100% | 102% |
| 6 | 103% | 100% | 106% | 98% | 98% |
| 9 | 101% | 104% | 97% | 104% | 101% |
| 12 | 107% | 111% | 113% | 109% | 105% |
| 24 | 92% | 118% | 110% | 110% | 105% |

**Table S16.** Cryoactivation of active renin concentration at 26°C

| storage time (hour) | relative value (v.s. 0 hour) | | | | |
|---|---|---|---|---|---|
| | plasma 11 | plasma 12 | plasma 13 | plasma 14 | plasma 15 |
| 0 | 100% | 100% | 100% | 100% | 100% |
| 1 | 104% | 104% | 95% | 95% | 95% |
| 3 | 100% | 106% | 102% | 98% | 98% |
| 6 | 104% | 102% | 102% | 91% | 91% |
| 9 | 87% | 98% | 86% | 87% | 87% |
| 12 | 91% | 98% | 88% | 86% | 85% |
| 24 | 91% | 102% | 88% | 83% | 82% |

**Table S17.** Influence of freeze-thaw cycles on active renin concentration

| number of freeze-thaw cycles | relative value (v.s. 0 cycle) | | |
|---|---|---|---|
| | plasma 16 | plasma 17 | plasma 18 |
| 0 | 100% | 100% | 100% |
| 1 | 103% | 109% | 92% |
| 3 | 101% | 91% | 100% |
| 5 | 98% | 90% | 95% |

**Table S18.** Baseline measurements of PAC and ARC by the CLEIA

| parameters | unilateral PA | bilateral PA | essential hypertension | *P* values |
|---|---|---|---|---|
| Number | 75 | 50 | 97 | |
| PAC (ng/dL) | 39.6 ± 21.9<br>36.3 (20.6-52.9) | 19.5 ± 9.1<br>18.0 (13.5-21.4) | 17.4 ± 9.8<br>15.5 (11.0-22.9) | < 0.05 * † ‡ |
| ARC (pg/mL) | 1.38 ± 1.07<br>1.05 (0.70-1.60) | 2.17 ± 1.83<br>1.50 (1.00-2.65) | 6.45 ± 5.24<br>5.10 (2.85-8.15) | < 0.05 † ‡ |
| $ARR_{ARC}$ (ng/dL per pg/mL) | 45.0 ± 41.7<br>31.1 (17.7-64.0) | 14.3 ± 8.4<br>12.5 (8.09-19.5) | 4.1 ± 3.4<br>2.69 (1.55-9.85) | < 0.05 * † ‡ |

Data were shown as mean ± standard deviation in an upper row and median (25-75$^{th}$ percentile) in a lower row. PAC indicates plasma aldosterone concentration; ARC, active renin concentration; CLEIA, chemiluminescent enzyme immunoassay; PA, primary aldosteronism; $ARR_{ARC}$, aldosterone-over-renin concentration ratio

* denotes statistical significance in comparison between unilateral and bilateral PA groups.

† denotes statistical significance in comparison between unilateral PA and essential hypertension groups.

‡ denotes statistical significance in comparison between bilateral PA and essential hypertension groups.

**Table S19.** Comparison of PAC measurements based on renal function

| parameters | total | CKD | non-CKD | *P* values |
|---|---|---|---|---|
| Number | 120 | 13 | 107 | |
| Serum creatinine (mg/dL) | 0.70 (0.59-0.86) | 1.07 (0.86-1.32) | 0.66 (0.58-0.85) | < 0.001 * |
| eGFR (mL/min/1.73m$^2$) | 76.8 (69.0-88.6) | 48.2 (43.1-58.7) | 79.2 (72.0-90.0) | < 0.001 * |
| LC-MS/MS PAC (ng/dL) | 45.6 (21.0-78.7) | 34.8 (18.7-86.5) | 46.7 (24.0-78.8) | *NS* |
| CLEIA PAC (ng/dL) | 57.6 (31.7-91.9) | 50.1 (30.9-96.2) | 59.4 (31.6-92.1) | *NS* |

Data were shown as and 25-75$^{th}$ percentile. PAC indicates plasma aldosterone concentration; CKD, chronic kidney disease; GFR, estimated glomerular filtration rate; LC-MS/MS, liquid chromatography tandem mass spectrometry; CLEIA; chemiluminescent enzyme immunoassay; NS, no significant difference. * denotes statistical significance in comparison between CKD and non-CKD.

**Table S20A.** Criterion values and coordinates of ROC analysis with $ARR_{ARC}$ for PA

| | criterion | sensitivity | 95% CI | specificity | 95% CI | +LR | -LR | +PV | -PV | cost |
|---|---|---|---|---|---|---|---|---|---|---|
| | ≥0.196 | 100.00 | 97.1 - 100.0 | 0.00 | 0.0 - 3.7 | 1.00 | | 56.3 | | 0.44 |
| | >3.073 | 100.00 | 97.1 - 100.0 | 56.70 | 46.3 - 66.7 | 2.31 | 0.00 | 74.9 | 100.0 | 0.19 |
| | >4.495 | 96.00 | 90.9 - 98.7 | 70.10 | 60.0 - 79.0 | 3.21 | 0.057 | 80.5 | 93.2 | 0.15 |
| | >5.794 | 92.00 | 85.8 - 96.1 | 75.26 | 65.5 - 83.5 | 3.72 | 0.11 | 82.7 | 88.0 | 0.15 |
| → | **>6.024** | **92.00** | **85.8 - 96.1** | **76.29** | **66.6 - 84.3** | **3.88** | **0.10** | **83.3** | **88.1** | **0.15** |
| | >7.705 | 88.80 | 81.9 - 93.7 | 82.47 | 73.4 - 89.4 | 5.07 | 0.14 | 86.7 | 85.1 | 0.14 |
| | >7.730 | 88.00 | 81.0 - 93.1 | 84.54 | 75.8 - 91.1 | 5.69 | 0.14 | 88.0 | 84.5 | 0.14 |
| | >9.030 | 84.00 | 76.4 - 89.9 | 89.69 | 81.9 - 94.9 | 8.15 | 0.18 | 91.3 | 81.3 | 0.14 |
| | >10.80 | 81.00 | 72.8 - 87.3 | 93.81 | 87.0 - 97.7 | 13.06 | 0.20 | 94.4 | 79.1 | 0.14 |
| | >11.13 | 81.00 | 72.8 - 87.3 | 94.85 | 88.4 - 98.3 | 15.68 | 0.20 | 95.3 | 79.3 | 0.13 |
| | >11.22 | 80.80 | 71.9 - 86.6 | 94.85 | 88.4 - 98.3 | 15.52 | 0.21 | 95.2 | 78.6 | 0.14 |
| | >11.74 | 76.00 | 67.5 - 83.2 | 94.85 | 88.4 - 98.3 | 14.74 | 0.25 | 95.0 | 75.4 | 0.16 |
| | >12.54 | 72.00 | 63.3 - 79.7 | 95.88 | 89.8 - 98.9 | 17.46 | 0.29 | 95.7 | 72.7 | 0.18 |
| | >13.71 | 68.80 | 59.9 - 76.8 | 97.94 | 92.7 - 99.7 | 33.37 | 0.32 | 97.7 | 70.9 | 0.19 |
| | >14.12 | 68.00 | 59.1 - 76.1 | 97.94 | 92.7 - 99.7 | 32.98 | 0.33 | 97.7 | 70.4 | 0.19 |
| | >15.60 | 64.00 | 54.9 - 72.4 | 97.94 | 92.7 - 99.7 | 31.04 | 0.37 | 97.6 | 67.9 | 0.21 |
| | >17.07 | 60.00 | 50.9 - 68.7 | 97.94 | 92.7 - 99.7 | 29.10 | 0.41 | 97.4 | 65.5 | 0.23 |
| | >18.08 | 56.00 | 46.8 - 64.9 | 98.97 | 94.4 - 100.0 | 54.32 | 0.44 | 98.6 | 63.6 | 0.25 |
| | >228.3 | 0.00 | 0.0 - 2.9 | 100.00 | 96.3 - 100.0 | | 1.00 | | 43.7 | 0.56 |

The arrow and bold indicate the cut-off value in screening PA from EH. $ARR_{ARC}$, aldosterone-over-renin concentration ratio; PA, primary aldosteronism; EH, essential hypertension; +PV, positive predictive value; -PV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio.

**Table S20B.** Criterion values and coordinates of ROC analysis with $ARR_{ARC}$ for APA

| | criterion | sensitivity | 95% CI | specificity | 95% CI | +LR | -LR | +PV | -PV | cost |
|---|---|---|---|---|---|---|---|---|---|---|
| | ≥0.196 | 100.00 | 95.2 - 100.0 | 0.00 | 0.0 - 3.7 | 1.00 | | 43.6 | | 0.564 |
| | >3.073 | 100.00 | 95.2 - 100.0 | 56.70 | 46.3 - 66.7 | 2.31 | 0.00 | 64.1 | 100.0 | 0.244 |
| | >7.614 | 96.00 | 88.8 - 99.2 | 82.47 | 73.4 - 89.4 | 5.48 | 0.049 | 80.9 | 96.4 | 0.116 |
| → | **>7.707** | **96.00** | **88.8 - 99.2** | **83.51** | **74.6 - 90.3** | **5.82** | **0.048** | **81.8** | **96.4** | **0.110** |
| | >10.79 | 93.33 | 85.1 - 97.8 | 93.81 | 87.0 - 97.7 | 15.09 | 0.071 | 92.1 | 94.8 | 0.0640 |
| | >11.13 | 93.33 | 85.1 - 97.8 | 94.85 | 88.4 - 98.3 | 18.11 | 0.070 | 93.3 | 94.8 | 0.0581 |
| | >11.40 | 92.00 | 83.4 - 97.0 | 94.85 | 88.4 - 98.3 | 17.85 | 0.084 | 93.2 | 93.9 | 0.0640 |
| | >12.35 | 88.00 | 78.4 - 94.4 | 95.88 | 89.8 - 98.9 | 21.34 | 0.13 | 94.3 | 91.2 | 0.0756 |
| | >14.80 | 84.00 | 73.7 - 91.4 | 97.94 | 92.7 - 99.7 | 40.74 | 0.16 | 96.9 | 88.8 | 0.0814 |
| | >16.12 | 80.00 | 69.2 - 88.4 | 97.94 | 92.7 - 99.7 | 38.80 | 0.20 | 96.8 | 86.4 | 0.0988 |
| | >17.16 | 76.00 | 64.7 - 85.1 | 97.94 | 92.7 - 99.7 | 36.86 | 0.25 | 96.6 | 84.1 | 0.116 |
| | >18.63 | 70.67 | 59.0 - 80.6 | 98.97 | 94.4 - 100.0 | 68.55 | 0.30 | 98.1 | 81.4 | 0.134 |
| | >19.30 | 70.67 | 59.0 - 80.6 | 100.00 | 96.3 - 100.0 | | 0.29 | 100.0 | 81.5 | 0.128 |
| | >228.2 | 0.00 | 0.0 - 4.8 | 100.00 | 96.3 - 100.0 | | 1.00 | | 56.4 | 0.436 |

The arrow and bold indicate the cut-off value in screening APA from EH. $ARR_{ARC}$, aldosterone-over-renin concentration ratio; APA, aldosterone-producing adenoma; EH, essential hypertension; +PV, positive predictive value; -PV, negative predictive value; +LR, positive likelihood ratio; -LR, negative likelihood ratio.

**Table S21A.** Sensitivity and specificity of screening for PA based on CLEIA measurements

| sensitivity (%) | plasma aldosterone concentration (ng/dL) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ARC (pg/mL) | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 10.0 | 11.0 | 12.0 | 13.0 | 14.0 | 15.0 | 16.0 | 17.0 | 18.0 | 19.0 |
| 1.0 | 80.8 | 80.0 | 80.0 | 80.0 | 77.6 | 76.8 | 76.0 | 76.0 | 73.6 | 68.8 | 64.8 | 61.6 | 57.6 | 56.0 | 48.8 |
| 2.0 | 80.8 | 80.0 | 80.0 | 80.0 | 77.6 | 76.8 | 76.0 | 76.0 | 73.6 | 68.8 | 64.8 | 61.6 | 57.6 | 56.0 | 48.8 |
| 3.0 | 91.2 | 90.4 | 90.4 | 90.4 | 86.4 | 85.6 | 84.8 | 84.8 | 81.6 | 76.8 | 72.8 | 68.8 | 64.0 | 61.6 | 55.2 |
| 4.0 | 96.8 | 96.0 | 96.0 | 96.0 | 92.0 | 91.2 | 90.4 | 90.4 | 87.2 | 82.4 | 78.4 | 74.4 | 69.6 | 67.2 | 59.2 |
| 5.0 | 97.6 | 96.8 | 96.8 | 96.8 | 92.8 | 92.0 | 91.2 | 91.2 | 88.0 | 83.2 | 79.2 | 75.2 | 70.4 | 68.0 | 60.0 |
| 6.0 | 99.2 | 98.4 | 98.4 | 98.4 | 94.4 | 93.6 | 92.8 | 92.8 | 89.6 | 84.8 | 80.8 | 76.8 | 71.2 | 68.8 | 60.8 |
| 7.0 | 99.2 | 98.4 | 98.4 | 98.4 | 94.4 | 93.6 | 92.8 | 92.8 | 89.6 | 84.8 | 80.8 | 76.8 | 71.2 | 68.8 | 60.8 |
| 8.0 | 99.2 | 98.4 | 98.4 | 98.4 | 94.4 | 93.6 | 92.8 | 92.8 | 89.6 | 84.8 | 80.8 | 76.8 | 71.2 | 68.8 | 60.8 |

| specificity (%) | plasma aldosterone concentration (ng/dL) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ARC (pg/mL) | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 10.0 | 11.0 | 12.0 | 13.0 | 14.0 | 15.0 | 16.0 | 17.0 | 18.0 | 19.0 |
| 1.0 | 97.9 | 97.9 | 97.9 | 97.9 | 97.9 | 97.9 | 97.9 | 97.9 | 99.0 | 99.0 | 99.0 | 99.0 | 99.0 | 100 | 100 |
| 2.0 | 90.0 | 90.0 | 90.7 | 90.7 | 90.7 | 90.7 | 91.8 | 91.8 | 93.8 | 93.8 | 93.8 | 95.9 | 95.9 | 96.9 | 96.9 |
| 3.0 | 76.3 | 76.3 | 77.3 | 77.3 | 77.3 | 77.3 | 78.4 | 78.4 | 81.4 | 81.4 | 81.4 | 86.6 | 88.7 | 91.8 | 92.8 |
| 4.0 | 65.0 | 66.0 | 67.0 | 67.0 | 67.0 | 68.0 | 69.1 | 70.1 | 73.2 | 75.3 | 75.3 | 80.4 | 82.5 | 85.6 | 87.6 |
| 5.0 | 57.7 | 58.8 | 60.8 | 61.9 | 61.9 | 62.9 | 63.9 | 67.0 | 71.1 | 73.2 | 73.2 | 78.4 | 80.4 | 84.5 | 86.6 |
| 6.0 | 49.5 | 50.5 | 52.6 | 54.6 | 54.6 | 56.7 | 57.7 | 60.8 | 64.9 | 69.1 | 71.1 | 76.3 | 78.4 | 83.5 | 85.6 |
| 7.0 | 38.1 | 39.2 | 43.3 | 46.4 | 47.4 | 51.5 | 53.6 | 57.7 | 62.9 | 67.0 | 70.1 | 75.3 | 77.3 | 82.5 | 84.5 |
| 8.0 | 32.0 | 33.0 | 37.1 | 40.2 | 47.4 | 45.4 | 47.4 | 52.6 | 58.8 | 62.9 | 70.1 | 71.1 | 74.2 | 79.4 | 81.4 |

PA indicates primary aldosteronism; CLEIA, chemiluminescent enzyme immunoassay; ARC, active renin concentration.

**Table S21B.** Sensitivity and specificity of screening for APA based on CLEIA measurements

| sensitivity (%) | plasma aldosterone concentration (ng/dL) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ARC (pg/mL) | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 10.0 | 11.0 | 12.0 | 13.0 | 14.0 | 15.0 | 16.0 | 17.0 | 18.0 | 19.0 |
| 1.0 | 42.7 | 42.7 | 42.7 | 42.7 | 42.7 | 42.7 | 42.7 | 42.7 | 41.4 | 41.4 | 38.7 | 36.0 | 34.7 | 33.4 | 30.7 |
| 2.0 | 82.7 | 82.7 | 82.7 | 82.7 | 82.7 | 82.7 | 81.4 | 81.4 | 78.7 | 78.7 | 74.7 | 72.0 | 69.4 | 66.7 | 61.4 |
| 3.0 | 93.4 | 93.4 | 93.4 | 93.4 | 92.0 | 92.0 | 90.7 | 90.7 | 90.7 | 90.7 | 84.0 | 81.4 | 78.7 | 76.0 | 70.7 |
| 4.0 | 100 | 100 | 100 | 100 | 98.7 | 98.7 | 97.4 | 97.4 | 94.7 | 94.7 | 90.7 | 88.0 | 85.4 | 81.4 | 76.0 |
| 5.0 | 100 | 100 | 100 | 100 | 98.7 | 98.7 | 97.4 | 97.4 | 94.7 | 94.7 | 90.7 | 88.0 | 85.4 | 81.4 | 76.0 |
| 6.0 | 100 | 100 | 100 | 100 | 98.7 | 98.7 | 97.4 | 97.4 | 94.7 | 94.7 | 90.7 | 88.0 | 85.4 | 81.4 | 76.0 |
| 7.0 | 100 | 100 | 100 | 100 | 98.7 | 98.7 | 97.4 | 97.4 | 94.7 | 94.7 | 90.7 | 88.0 | 85.4 | 81.4 | 76.0 |
| 8.0 | 100 | 100 | 100 | 100 | 98.7 | 98.7 | 97.4 | 97.4 | 94.7 | 94.7 | 90.7 | 88.0 | 85.4 | 81.4 | 76.0 |

| specificity (%) | plasma aldosterone concentration (ng/dL) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| ARC (pg/mL) | 5.0 | 6.0 | 7.0 | 8.0 | 9.0 | 10.0 | 11.0 | 12.0 | 13.0 | 14.0 | 15.0 | 16.0 | 17.0 | 18.0 | 19.0 |
| 1.0 | 98.0 | 98.0 | 98.0 | 98.0 | 98.0 | 98.0 | 98.0 | 98.0 | 99.0 | 99.0 | 99.0 | 99.0 | 99.0 | 100 | 100 |
| 2.0 | 89.7 | 89.7 | 90.8 | 90.8 | 90.8 | 90.8 | 91.8 | 93.9 | 93.9 | 93.9 | 93.9 | 95.9 | 95.9 | 97.0 | 97.0 |
| 3.0 | 76.3 | 76.3 | 77.4 | 77.4 | 77.4 | 77.4 | 78.4 | 78.4 | 81.5 | 81.5 | 81.5 | 86.6 | 88.7 | 91.8 | 92.8 |
| 4.0 | 65.0 | 66.0 | 67.1 | 67.1 | 67.1 | 68.1 | 69.1 | 70.2 | 73.2 | 75.3 | 75.3 | 80.5 | 82.5 | 85.6 | 87.7 |
| 5.0 | 57.8 | 58.8 | 60.9 | 61.9 | 61.9 | 62.9 | 64.0 | 67.1 | 71.2 | 73.2 | 73.2 | 78.4 | 80.5 | 84.6 | 86.6 |
| 6.0 | 49.5 | 50.6 | 52.6 | 54.7 | 54.7 | 56.8 | 57.8 | 60.9 | 65.0 | 69.1 | 71.2 | 76.3 | 78.4 | 83.6 | 85.6 |
| 7.0 | 38.2 | 39.2 | 43.3 | 46.4 | 47.5 | 51.6 | 53.7 | 57.8 | 62.9 | 67.1 | 70.2 | 75.3 | 77.4 | 82.5 | 84.6 |
| 8.0 | 32.0 | 33.0 | 37.2 | 40.3 | 47.5 | 47.5 | 47.5 | 52.6 | 58.8 | 62.9 | 70.2 | 71.2 | 74.3 | 79.4 | 81.5 |

APA indicates aldosterone-producing adenoma; CLEIA, chemiluminescent enzyme immunoassay; ARC, active renin concentration.

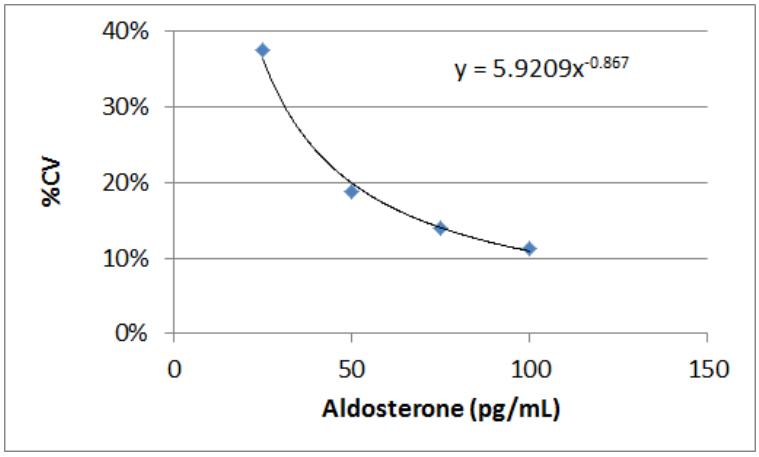


**Figure S1.** Limit of quantification in CLEIA of PAC. CLEIA indicates chemiluminescent enzyme immunoassay; PAC, plasma aldosterone concentration.

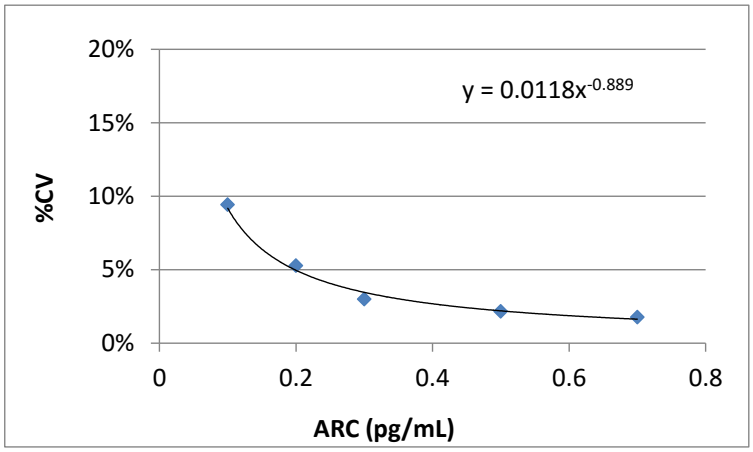


**Figure S2.** Limit of quantification in CLEIA of ARC. CLEIA indicates chemiluminescent enzyme immunoassay; ARC, active renin concentration.

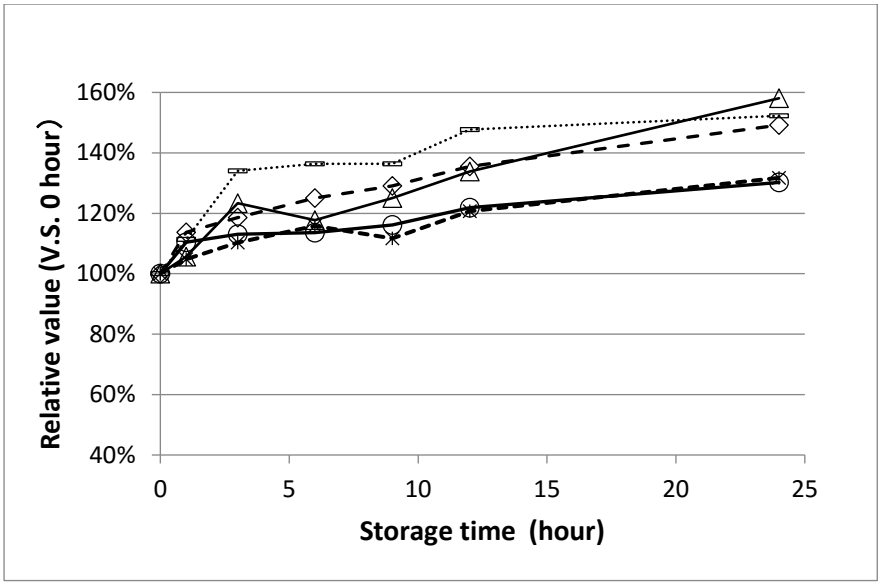


**Figure S3.** Influence of storage temperature on stability of ARC at 0°C. ARC indicates active renin concentration.

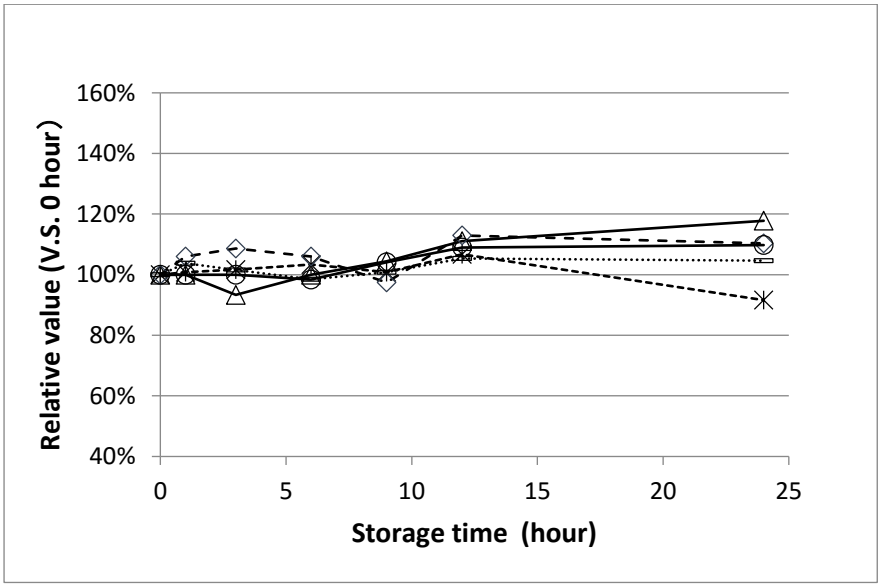


**Figure S4.** Influence of storage temperature on stability of ARC at 5°C. ARC indicates active renin concentration.

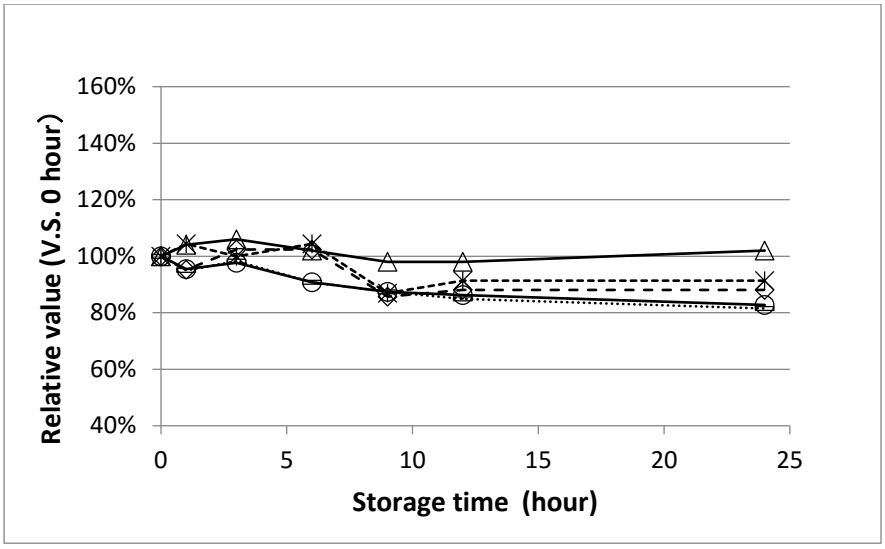


**Figure S5.** Influence of storage temperature on stability of ARC at 26°C. ARC indicates active renin concentration.

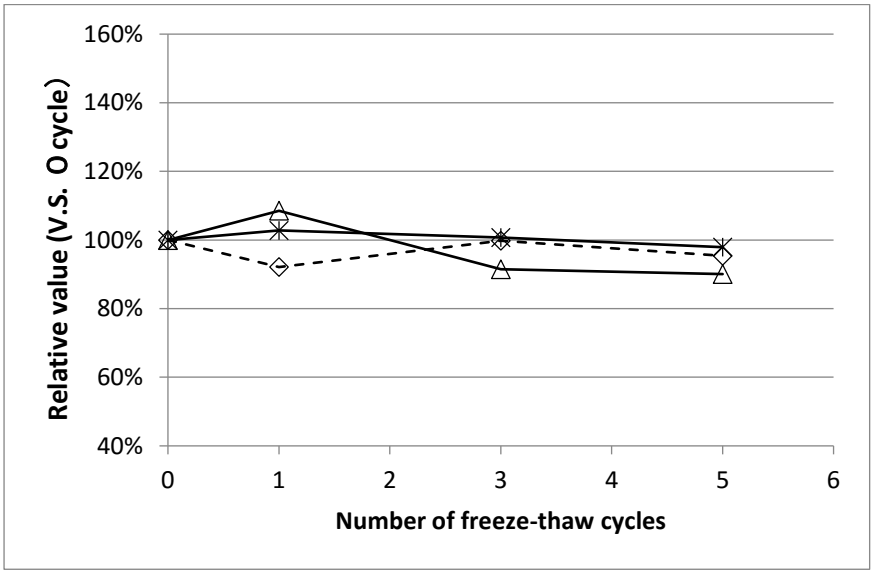


**Figure S6.** Influence of freeze-thaw cycles on stability of ARC. ARC indicates active renin concentration.

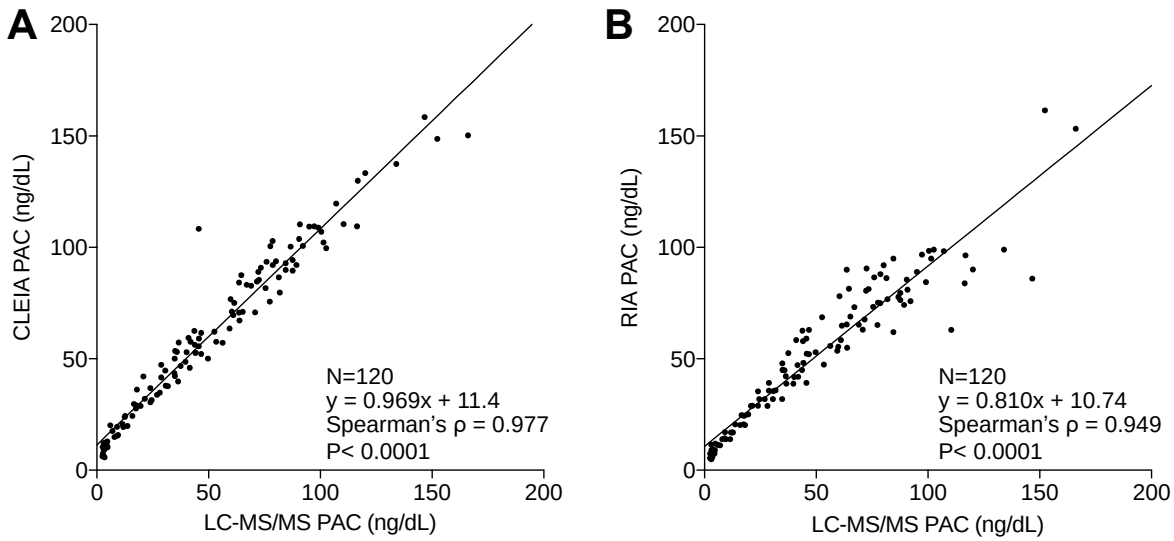


**Figure S7.** Correlation of PAC between CLEIA and LC-MS/MS (A). Correlation of PAC between RIA and LC-MS/MS (B). PAC indicates plasma aldosterone concentration; CLEIA, chemiluminescent enzyme immunoassay; LC-MS/MS, liquid chromatography tandem mass spectrometry; RIA, radioimmunoassay.

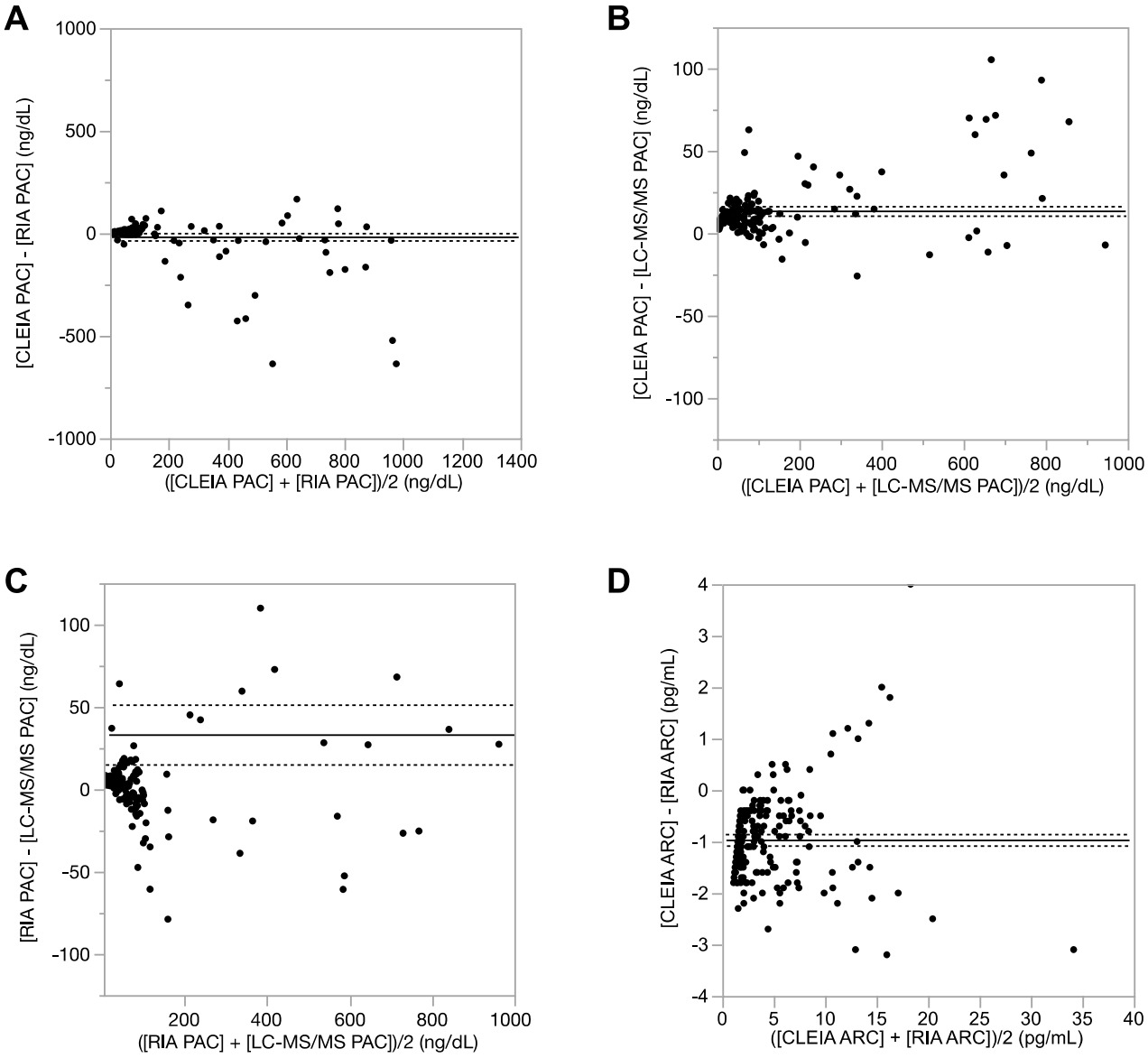


**Figure S8.** Bland-Altman plot of CLEIA and RIA measurements of aldosterone (A), that of CLEIA and LC-MS/MS measurements of aldosterone (B), that of RIA and LC-MS/MS measurements of aldosterone (C) and that of CLEIA and RIA measurements of renin (D). RIA indicates radioimmunoassay; PAC, plasma aldosterone concentration; CLEIA, chemiluminescent enzyme immunoassay; LC-MS/MS, liquid chromatography tandem mass spectrometry; ARC, active renin concentration.

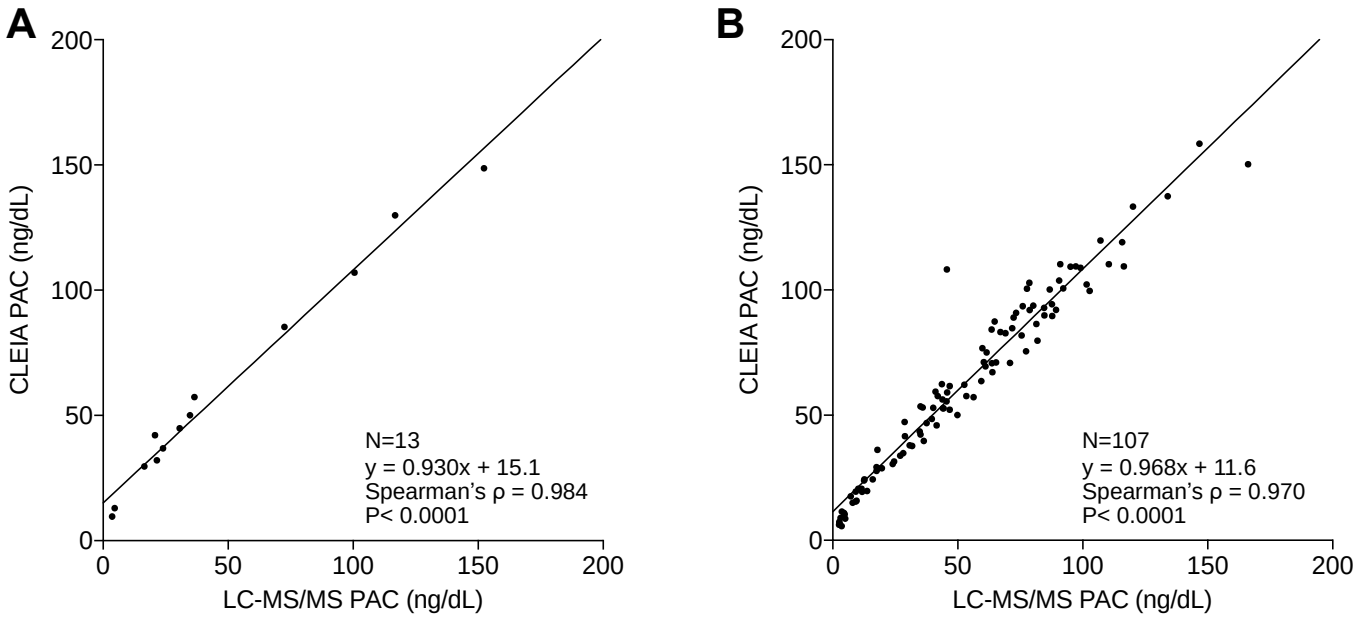


**Figure S9.** Correlation of PAC measurements between CLEIA and LC-MS/MS in those with renal insufficiency (A) and without renal insufficiency (B). PAC indicates plasma aldosterone concentration; CLEIA, chemiluminescent enzyme immunoassay; LC-MS/MS, liquid chromatography tandem mass spectrometry.

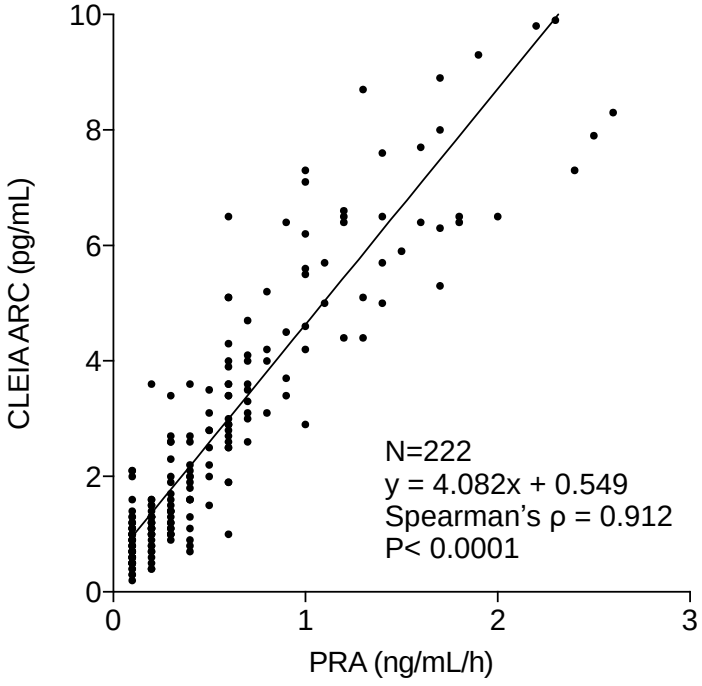


**Figure S10.** Correlation of measurements between CLEIA ARC and PRA. CLEIA indicates chemiluminescent enzyme immunoassay; ARC, active renin concentration; PRA, plasma renin activity.

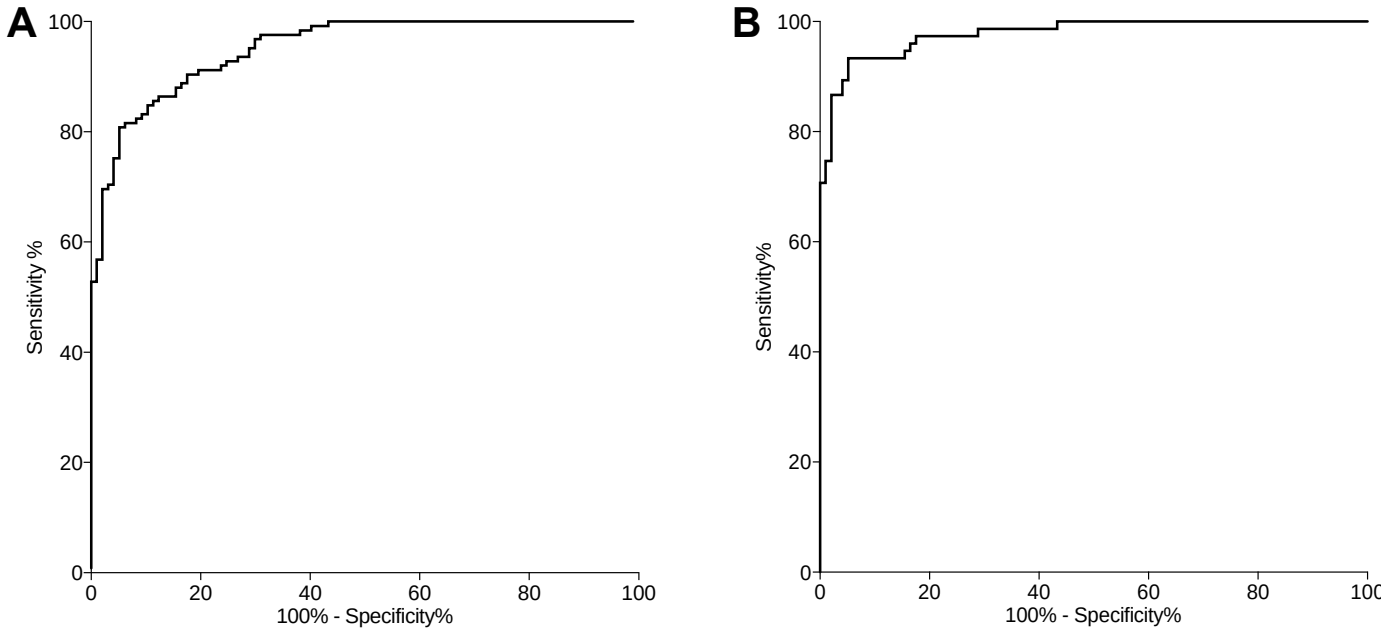


**Figure S11.** Receiver operating characteristic analysis of $ARR_{ARC}$ as a screening index for PA from EH (A) and APA from EH (B), respectively. $ARR_{ARC}$ indicates aldosterone-over-renin concentration ratio; PA, primary aldosteronism; EH, essential hypertension; APA, aldosterone-producing adenoma.