Immuno-MALDI Assay for Plasma Renin Activity: Proof of Principle for a Translatable Proteomic Assay Without Chromatography

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Overview:

- Plasma Renin Activity (PRA) has a long history and strong literature backing for screening and diagnosis of Primary Aldosteronism (PA)
- Classically, PRA assays require a radioimmonassay (RIA) for Angiotensin-I (Ang-I).
- Clinical laboratories are being encouraged to phase out radioisotopic methods where possible
- We propose a MALDI based method for the measurement of Ang-I, and the determination of PRA
- Co-capture of natural Ang-I and SIS Ang-I allows for the accurate quantitation of natural Ang-I present in the sample
- Our method has been compared to an RIA in clinical production

Introduction:

Measurement of plasma renin activity (PRA) is essential in the screening and diagnostic process for primary aldosteronism (PA) and a number of other diseases of the RAAS pathway[1]. Although automated chemiluminescent sandwich assays for the renin molecule now exist[2], their analytical performance in low-renin hypertensive states is poor and so PRA remains the gold standard for the evaluation of the RAAS pathway in this context[2,3]. With the technical ease and substantial clinical success of MALDI approaches to bacterial identification, we have set out to develop a quantitative clinical assay for a MALDI platform.

PRA assays work by taking EDTA plasma collected in appropriate conditions (usually a pre-chilled tube, cold centrifugation, and snap freezing until analysis), incubating in buffering conditions (pH 5.5-7.4) at 37°C and Ang-I is then generated from renin activity on endogenous angiotensinogen substrate. Concomitant incubation at 0°C allows for subtraction of the background Ang-I concentration.

$$\text{Angiotensinogen} \xrightarrow{\text{Renin}} \text{Angiotensin I}$$

Plasma renin activity is then calculated by:

$$PRA = ([\text{Ang-I}]_{37^\circ C} - [\text{Ang-I}]_{0^\circ C}) / \Delta t$$

We present an iMALDI based assay for the measurement of PRA which correlates with a clinically-implemented RIA, while affording the accuracy and specificity of mass spectrometry and eliminating the use of radioisotopic reagents.

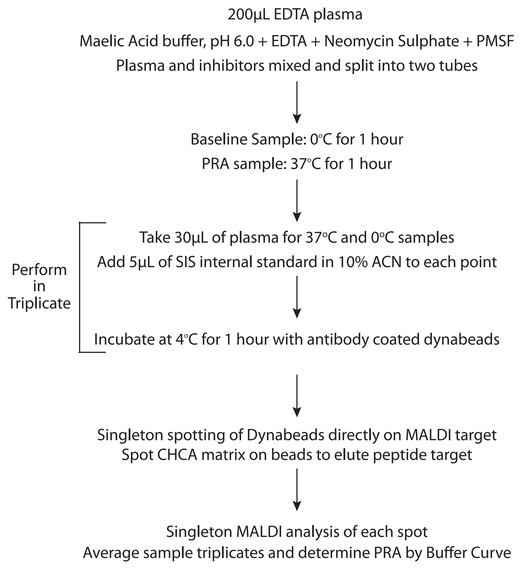
Methods:

The iMALDI approach utilizes the capture of Ang-I by polyclonal antibody (Santa Cruz) coated Dynabeads (Invitrogen) which are deposited directly on a MALDI target for MS analysis. This method is sensitive enough to only require a 1 h Ang-I generation step, followed by a 1 h antibody capture. Detection by MALDI MS is rapid, allowing potential for high throughput analysis after sample preparation is complete. EDTA plasma samples from routine outpatient collections from the St. Paul's Hospital PA screening program were run by:

- RIA (1 h incubation at 37°C with 4°C blanking, pH=7.4 with antibody capture and 48 h RIA step, Normal Range: 0.05-0.55 ng/L/s)
- LC-MS/MS (3 h incubation at 37°C with 4°C blanking, pH=6.0 with immediate LC-MS/MS analysis on the ABSCIEX API5000, Normal Range: 0.10-1.10 ng/L/s)
- iMALDI (1 h incubation at 37°C with 4°C blanking, pH=6, 1 h antibody capture, immediate MALDI analysis on the ABSCIEX AB4800)

Calibrators for all three PRA methods were verified against the NIBSC reference material for Angiotensin-I.
(ref:http://www.nibsc.ac.uk/documents/ifu/86-536.pdf).

Workflow:



Results:

We have developed and evaluated three approaches for iMALDI screening for the determination of PRA. The first method utilizes two standard curves, one for samples incubated at 0°C and one for samples incubated at 37°C. From the slopes of the standard curves, we can determine the x-intercepts, with the difference between the two x-intercepts indicating the amount of Ang-I generated, and therefore the PRA. We analyzed 25 clinical samples with this 2-curve method, and obtained a good correlation between the iMALDI and RIA results (R^2=0.8107), and iMALDI and LC-MS/MS results (R^2=0.8091).

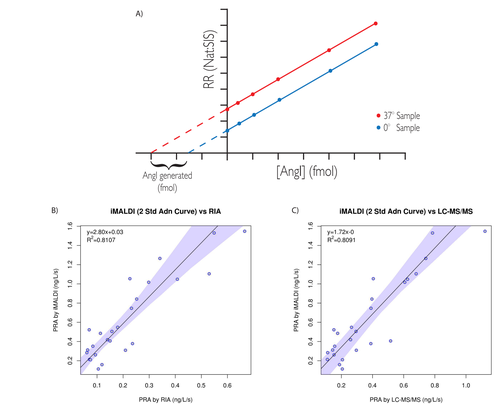


Figure 1: A) Illustration of the 2 standard curve iMALDI method. B) RIA vs 2 standard curve iMALDI method PRA results (n=25). C) LC-MS/MS vs 2 standard curve iMALDI method PRA results (n=25).

As the two standard curves have an evidently identical slope, the second iMALDI method increases throughput by only generating the 0° standard curve, and performing a triplicate analysis of the sample incubated at 37°C. The slope of the single standard curve is used for both the 0°C and 37°C measurements to determine the PRA, as described above. Twenty-five clinical samples were analyzed this way, also showing strong correlation with RIA and LC-MS/MS (R^2=0.8760 and R^2=0.8562, respectively).

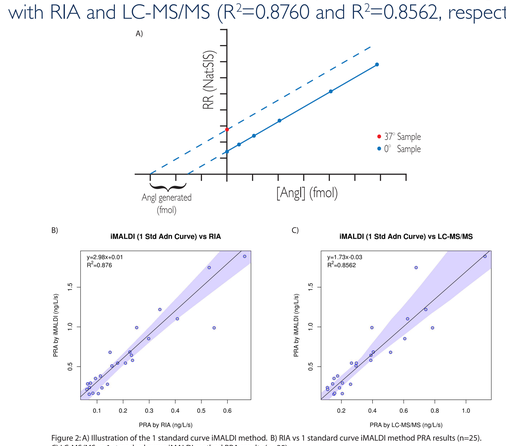


Figure 2: A) Illustration of the 1 standard curve iMALDI method. B) RIA vs 1 standard curve iMALDI method PRA results (n=25). C) LC-MS/MS vs 1 standard curve iMALDI method PRA results (n=25).

Finally, in order to develop a clinically useful assay capable of handling a high sample throughput, we developed an iMALDI assay using triplicate analysis of a single 0°C and 37°C degree point, and determined its PRA by comparing it to a standard curve for Ang-I captured in buffer. This iMALDI method is the closest to the RIA method and showed stronger correlation for 64 clinical samples with both RIA and LC-MS/MS (R^2=0.9412 and R^2=0.9471, respectively).

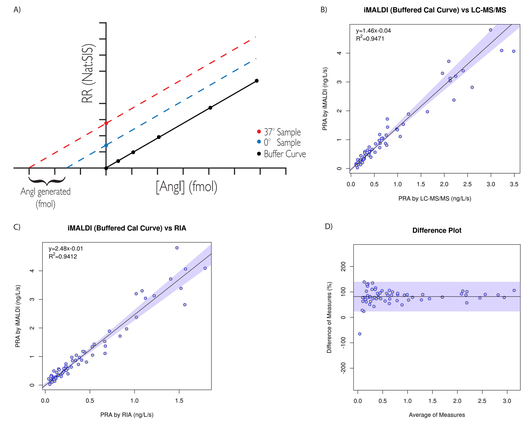


Figure 3: A) Illustration of the buffer standard curve iMALDI method. B) LC-MS/MS vs buffer standard curve iMALDI method PRA results (n=64). C) RIA vs buffer standard curve iMALDI method PRA results (n=64).D) Bland Altman plot of the average vs the difference of RIA vs the buffer standard curve iMALDI method (n=64).

The slope of 2-3 seen in all of the RIA regression lines is partially attributable to the difference in pH between the RIA method[4] (pH 7.4) and the iMALDI method (pH 6.0), as plasma renin is approximately twice as active at pH 6 than physiological pH. The LC-MS/MS method was also performed at the lower pH (pH 6) giving a regression slope closer to 1 – though predictably not exactly 1 since PRA should be higher for shorter incubations due to higher endogenous substrate concentrations which are gradually depleted over longer incubations..

Conclusions:

With these results, we believe we have achieved an iMALDI method for the determination of PRA that will be clinically useful for PRA measurment after automated pipetting and automated MALDI spotting have been implemented.

References:

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