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

Alex Camenzind[1], Grace Van DerGugten[2], **Daniel T. Holmes[2]**, Christoph Borchers[1]

1. University of Victoria Proteomics Centre, Vancouver Island Technology Park, 3101-4464 Markham St, Victoria, BC Canada, V8Z 7X8
2. St. Paul's Hospital, 1081 Burrard St, Vancouver, BC Canada, V6Z 1Y6

The renin-angiotensin-aldosterone system (RAAS) plays an essential role in maintaining plasma volume and arterial blood pressure. 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. Although automated chemiluminescent sandwich assays for the renin molecule now exist, 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. Clinical laboratories have traditionally used radioimmunoassay (RIA) to determine PRA through determination of angiotensin-I after plasma incubation in appropriate buffering conditions for 3h.

With the advent of homogenous chemiluminescent and stable-isotopic MS methods for small molecules, many clinical labs have been encouraged to move away from radioisotopic methods where possible. Though MRM-based LC-MS/MS approaches for PRA are an attractive alternative, many clinical labs have shied away from LC-MS/MS entirely because of the technical demand that HPLC places on technologists. 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.

The iMALDI approach utilizes the capture of angiotensin I by antibody 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 angiotensin-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 64 routine outpatient collections from the St. Paul's Hospital PA screening program were run by: RIA[1] (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), by LC-MS/MS[2] (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: 0.10-1.00 ng/L/s), and by 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.

The regression relationship between iMALDI and RIA was iMALDI=2.48×RIA-0.01 (Passing Bablok, n=64, R^2=0.9412) and LC-MS/MS was iMALDI=1.46×LC-MS/MS-0.04 ng/L/s (Passing Bablok, n=64, R^2=0.9471)

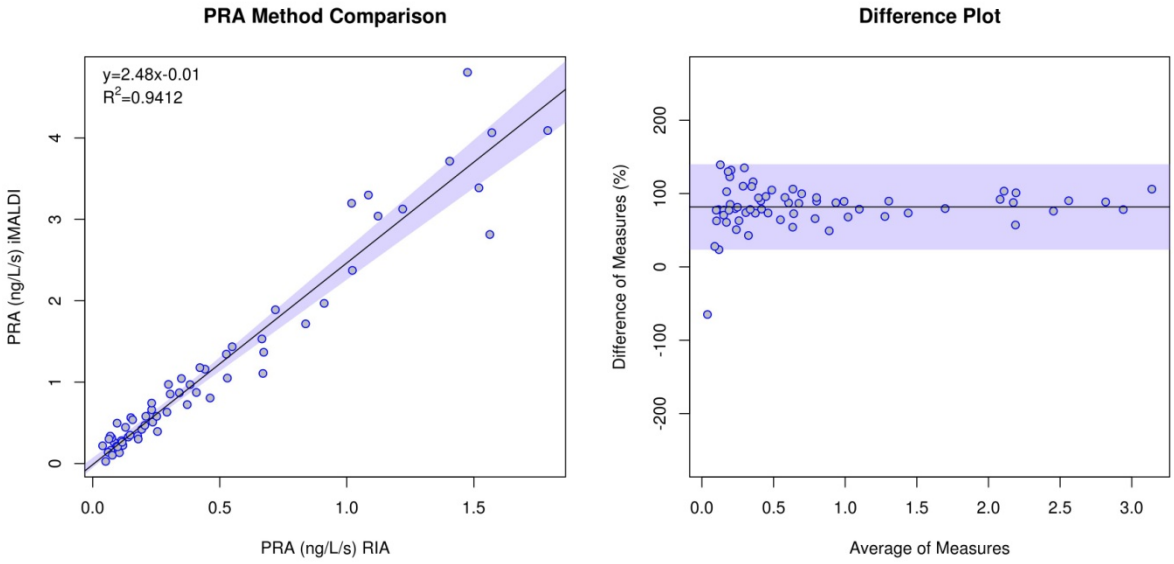

Figure 1: Method comparison and difference plots between PRA by iMALDI and RIA. To convert ng/L/s to ng/mL/h, multiply by 3.6. Note that RIA was buffered to pH=7.4 which causes numerical results to be ~50% lower than commercial assays buffering to pH=6.0.

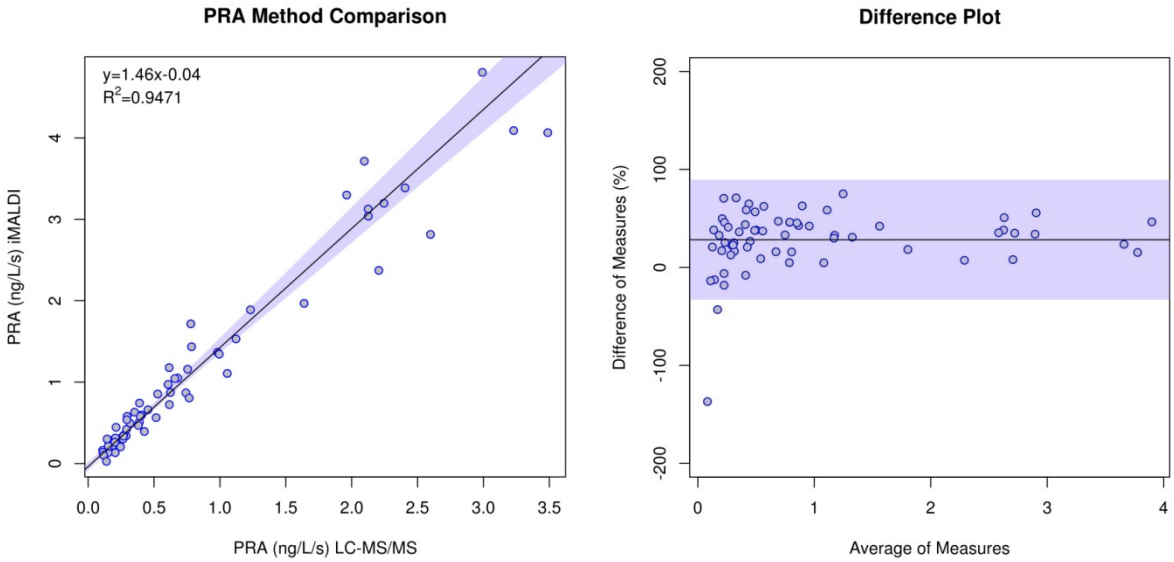

Figure 2: Method comparison and difference plots between PRA by iMALDI and LC-MS/MS. To convert ng/L/s to ng/mL/h, multiply by 3.6.

Correlation between methods was outstanding providing proof concept that the iMALDI could be suited for clinical use in quantitative testing. Differences in numerical results by different PRA methods are related to differences in buffering conditions and duration of incubation. This is a well-known phenomenon[3]. Refinement of the iMALDI workflow by using automated pipetting, implementation of a routine calibrator (as opposed to standard addition curves), and automated MALDI plate spotting will facilitate a transition to clinical setting.

1. Poulsen, K., and J. Jorgensen. "An easy radioimmunological microassay of renin activity, concentration and substrate in human and animal plasma and tissues based on angiotensin I trapping by antibody." Journal of Clinical Endocrinology & Metabolism 39.5 (1974): 816-825.

2. Van Der Gugten, J. Grace, and Daniel T. Holmes. "Plasma Renin Activity by Tandem Mass Spectrometry Employing Analyte Immunoprotection." ASMS Conference, Vancouver, 2012.

3. Sealey, Jean E., and John H. Laragh. "Radioimmunoassay of plasma renin activity." Seminars in nuclear medicine. Vol. 5. No. 2. WB Saunders, 1975.