ARE ALDOSTERONE ASSAYS BY LC-MS/MS HARMONISED IN AUSTRALIA?

ZX Lu,[1,2] J Liao,[1] K Liu,[1] K Mantik,[3] K Hoad,[4] B McWhinney,[5] R Sahertian,[3] GRD Jones,[6] M Gillett,[4] J Ungerer,[5] AR Horvath[3]

[1]Monash Health Pathology, VIC; [2]Department of Medicine, Monash University, VIC; [3]NSW Health Pathology, NSW; [4]PathWest Laboratory Medicine WA; [5]Pathology Queensland, QLD; [6]SydPath, St Vincent's Hospital, NSW.

Zhong.lu@monashhealth.org

Introduction

Accurate measurement of aldosterone is crucial for the investigation of primary aldosteronism. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) provides better selectivity over immunoassays by avoiding cross-reactivity with other steroid compounds that have similar structure to aldosterone. We compared the analytical performance of LC-MS/MS aldosterone assays in Australia in view of harmonising reference intervals and diagnostic decision limits.

Methods

Aliquots from 30 de-identified clinical samples with aldosterone concentrations ranging from 27 - 2600 pmol/L, were sent to four laboratories.

LCMS/MS analysers used:

- Lab1: Sciex QTRAP 5500; Lab2: Sciex QTRAP 6500+
- Lab3: Waters Xevo TQ-S micro; Lab4: Waters Xevo TQ-S.

Calibrators:

Laboratories used either commercial calibrators (Chromsystems) or in-house calibrators prepared by spiking aldosterone standard (Cerilliant) into stripped serum (Golden West).

Acceptance criteria :

Mean results of the four methods were used for comparison. RCPAQAP analytical performance specifications (APS) (±24 up to 160; ±15% >160 pmol/L) were used as acceptance limits.

The precision goal for analytical CV (Cv_a) was defined using within-subject biological variation (CV_i) of 36.6%[1]: optimal = 0.25xCV_i =9.1%; desirable = 0.5xCV_i =18.3%; minimal = 0.75xCV_i =27.4%.[2]

Precision of the assays:

Cv_a was calculated for each sample using results from the four laboratories. Results from one sample with an interference peak was excluded.

A precision profile was generated by plotting Cv_a values for each sample against the corresponding aldosterone concentrations.

Results

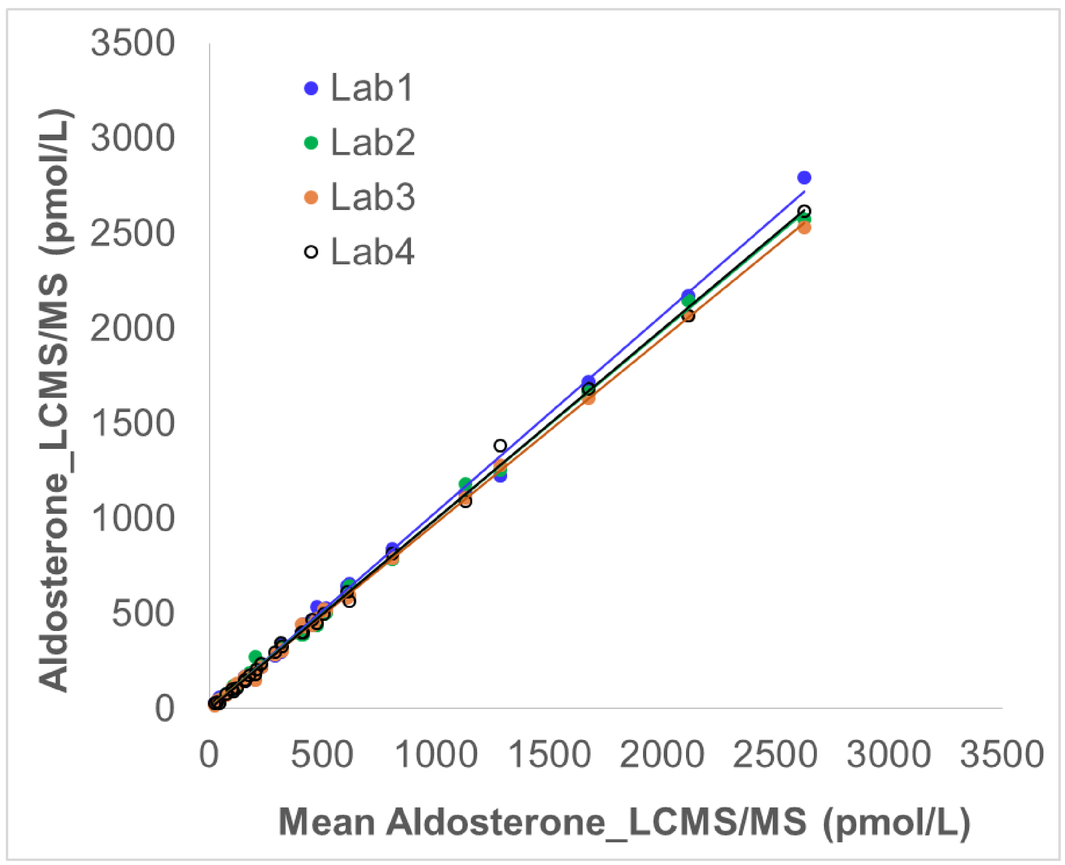

Fig 1. Patient correlation of aldosterone by LCMS/MS results

Compared to the mean, r^2>0.997 was achieved by all laboratories. The Passing-Bablok equation for each laboratory compared to the mean was:

Lab1 = 1.028 x mean -2.6;
Lab2 = 0.975 x mean +5.2;
Lab3 = 0.997 x mean +2.2;
Lab4 = 0.970 x mean +2.2.

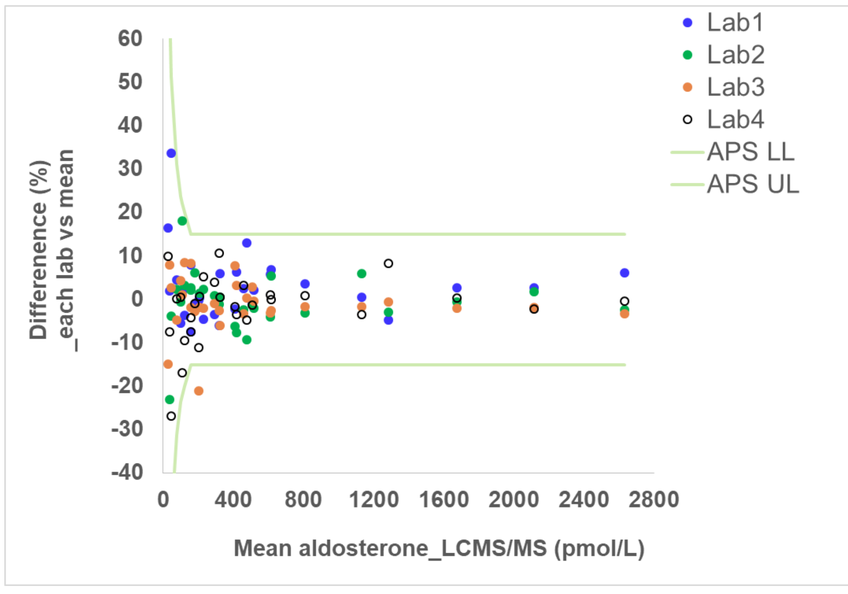

Fig 2. Difference plot of all aldosterone by LCMS/MS results vs mean

All results (except two) were within the mean ± APS for each sample. One of the outliers was found to have a method-dependent interference peak.

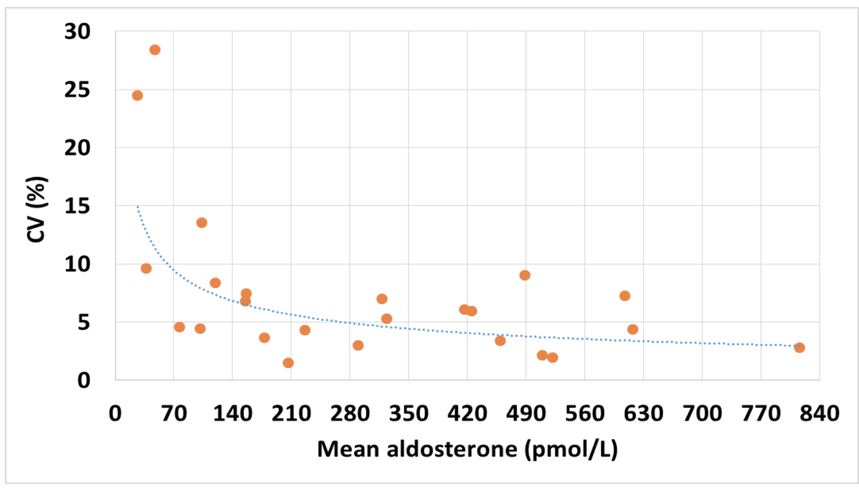

Fig 3. Precision profile using combined data from all laboratories

For all the samples (except one) with aldosterone >70 pmol/L the Cv_a achieved the optimal precision goal of ≤9.1%, adding only less than 3% of variability in test results seen in the patient, according to the chart for Result Variability vs Ratio of Analytical Imprecisions to Within-subject Biological Variation by Callum Fraser.[2]

At around_160 pmol/L, a proposed cut-off for positive saline suppression test in the Harmonisation of Endocrine Dynamic Testing-Adult (HEDTA) protocol,[3] the Cv_a was ~7%.

Cv_a for aldosterone < 70 pmo/L was higher (10-28%) but still met at least the minimal precision goal.

Discussion and Conclusion

This study in commutable specimens showed that there is good agreement in aldosterone results by the LC-MS/MS methods from four different laboratories. This paves the way for moving towards national harmonisation of the reference intervals and clinical decision limits for aldosterone by LC-MS/MS in Australia.

Further collaboration is required for all the laboratories to work together to harmonise their method, reference intervals and decision limits used in the diagnostic pathway of primary aldosteronism.

References:

1. EFLM Biological Variation Database: https://biologicalvariation.eu/
2. Fraser CG. 2001, Biological variation from principles to practice. AACC Press, Washington. Page 51.
3. Chiang C et al. 2021, Harmonisation of Endocrine Dynamic Testing-Adult (HEDTA) protocol. Available at https://www.aacb.asn.au/documents/item/5429.