Addition Of Pyridoxal Phosphate Cofactor To Aminotransferase Reagent Produces A Clinically Significant Increase In Measurement Of ALT And AST For Patients Who Are Deficient In The Cofactor

J. Lawrence, M. Saleem, P. Coates, T. Petrou, T. Biszak

1 Automated Biochemistry, SA Pathology, Adelaide, South Australia.
2 Chemical Pathology, SA Pathology, Adelaide, South Australia.

Introduction
Pyridoxal phosphate (PSP or vitamin B6) is a cofactor that is required for the full catalytic activity of alanine transferase (ALT) and aspartate transferase (AST). The concentration of PSP in serum varies considerably among individuals and some patient groups are known to be deficient in PSP. The International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommends addition of PSP to ALT and AST reagents to ensure measurement of full enzymatic activity (IFCC method). The current Siemens ADVIA ALT and AST methods are modified from this method due to the omission of PSP in the reagent (modified IFCC method) and this may underestimate ALT and AST activity in PSP deficient patients.

Aminotransferases require all aspects of the assay to be present in the right conditions for the assays to work at optimal level. AST: measured at 340/410nm
ALT + PSP  -->  Activated AST
L-Aspartate + α-ketoglutarate  -->  Oxalacetaate + L-Glutamate
Oxalacetate + NADH  →  Malate Dehydrogenase  →  Malate + NAD

ALT: measured at 340/410nm
ALT + PSP  ---->  Activated ALT
L-Alanine + α-Ketoglutarate  -->  Pyruvate + L-Glutamate
Pyruvate + NADH  →  Lactate Dehydrogenase  →  Lactate + NAD

Method
We measured ALT and AST on 320 samples using both the IFCC recommended method and the current Siemens modified IFCC method. We included patients who are more likely to be PSP deficient (ICU patients, patients from drug and alcohol withdrawal services, pregnant patients, transplant patients) as well as routine hospital ED and community patients. Method comparison analysis was done using Bland-Altman difference for bias and Passing and Bablok regression.

Results
There was a significant negative bias noted for the modified IFCC ALT method compared with the IFCC recommended measurement: Mean bias -4.8 U/L (95% CI -5.8 to -3.8 ). Passing and Bablok evaluation gave the regression equation, y =0.91x + 0.07, where y = modified IFCC method and x = IFCC method with a slope of 0.92 (95% CI 0.919 to 0.927).

There was a significant negative bias noted for the modified IFCC AST method compared with the IFCC recommended measurement: Mean bias -8.0 U/L (95% CI -9.7 to -6.3). Passing and Bablok evaluation gave the regression equation, y =0.86x + 0.71, where y = modified IFCC method and x = IFCC method with a slope of 0.89 (95% CI 0.81 to 0.91).

The results were compared to the RCPA Allowable Limits of Performance (ALP). Both assays have an ALP of ± 5 -40 UL and 12% -40UL/L. For the AST method there were 33.75% of results that were outside of the ALP when the two methods were compared. ALT had 12.8% that were outside the ALP when comparing the methods. This significant difference supports the literature, which states that PSP is required for the activation of AST and ALT and without this additive the assays underestimate the aminotransferase activity. Hence, the patients that had differences greater than the ALP could be considered deficient in vitamin B6. Some of the patients considered deficient, were from: Intensive Care Unit, Oncology Unit, Transplant Unit, Drug and Alcohol Withdrawal Service. These patients all had either a decrease in function of their liver or were malnourished.

Conclusion
We found a significant underestimation of ALT and AST using the current Siemens ADVIA ALT/AST methods which could have impact on patient groups who are likely to be PSP deficient. Based on this data, our laboratory has implemented the IFCC recommended ALT and AST methods with PSP on the Siemens ADVIA 2400 analysers. Our recommendation is for other laboratories that provide a service to patients who may be PSP deficient, to consider adopting the IFCC recommended method for ALT and AST.

Acknowledgements
Siemens Healthcare for their help and supply of reagents during this study.