A-132

Next generation ammonia reagent with improved reagent composition and assay performance

Sandhya Nair, Sandy Chandler, Lorna White, and Robert Acorn
SEKISUI Diagnostics P.E.I. Inc., Charlottetown, PEI, Canada

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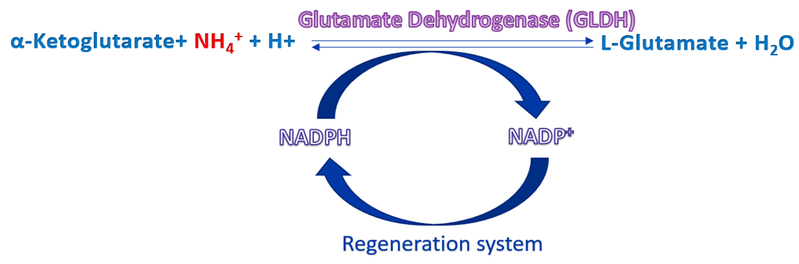
Introduction

Plasma Ammonia is generated primarily in the gastrointestinal tract by metabolism of proteins and other nitrogenous compounds. Ammonia is converted to urea by Krebs-Henseleit Urea cycle in the liver hepatocytes and so rendered non-toxic. Elevated ammonia is observed in severe liver failure, Reye's Syndrome, viral hepatitis, or cirrhosis. Therefore, elevated ammonia levels can aid in diagnosis of liver failure or hepatic encephalopathy. The reliable and accurate quantification of ammonia is influenced by assay, reagent, and sample management. Current commercially available GLDH enzyme-based ammonia assay reagents experience high bias between matched Lithium Heparin and K2-EDTA samples, poor accuracy, imprecision at the low end, large HIL (hemolysis, icterus and lipemia) interferences and shorter shelf life. To improve upon the listed deficiencies, we developed an improved GLDH based ammonia assay in a two-part format with improved reagent composition.

Test principle

Figure 1. Assay principle



The decrease in NADPH is directly related to the concentration of ammonia (NH_4^+) in the sample and can be conveniently measured by determining the decrease in absorbance of NADPH at 340nm. The regeneration system maintains a suitable concentration of NADPH for the shelf-life of reagent.

Methods

Next Generation ammonia reagent in 2-part format R1 and R2 were assessed for accuracy, precision, interference by HIL, reportable range, sensitivity (LoB, LoD, LoQ), shelf life and matched matrix comparison (Lithium Heparin vs K2-EDTA). All testing were performed using Li Heparin plasma samples on the Beckman Coulter DxC 700 AU® chemistry analyzer.

Results

Reportable range

Recovery of ACS Verichem ammonia standards confirmed accuracy of the assay. Plasma linearity (CLSI-EP06) covered the reportable range of 12 to 1017 µmol/L. Maximum deviation for mean recovery was ± 6 µmol/L or ± 10%.

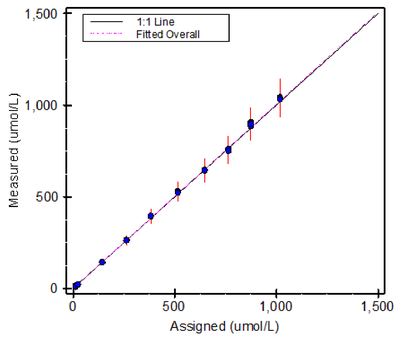


Figure 2. Next generation ammonia assay with 9 levels of plasma covering the reportable range

Slope	Intercept	Bias
1.006	-1.70	3%

Beckman Coulter DxC 700 AU® is a registered trademark of Beckman Coulter, Inc.

Precision

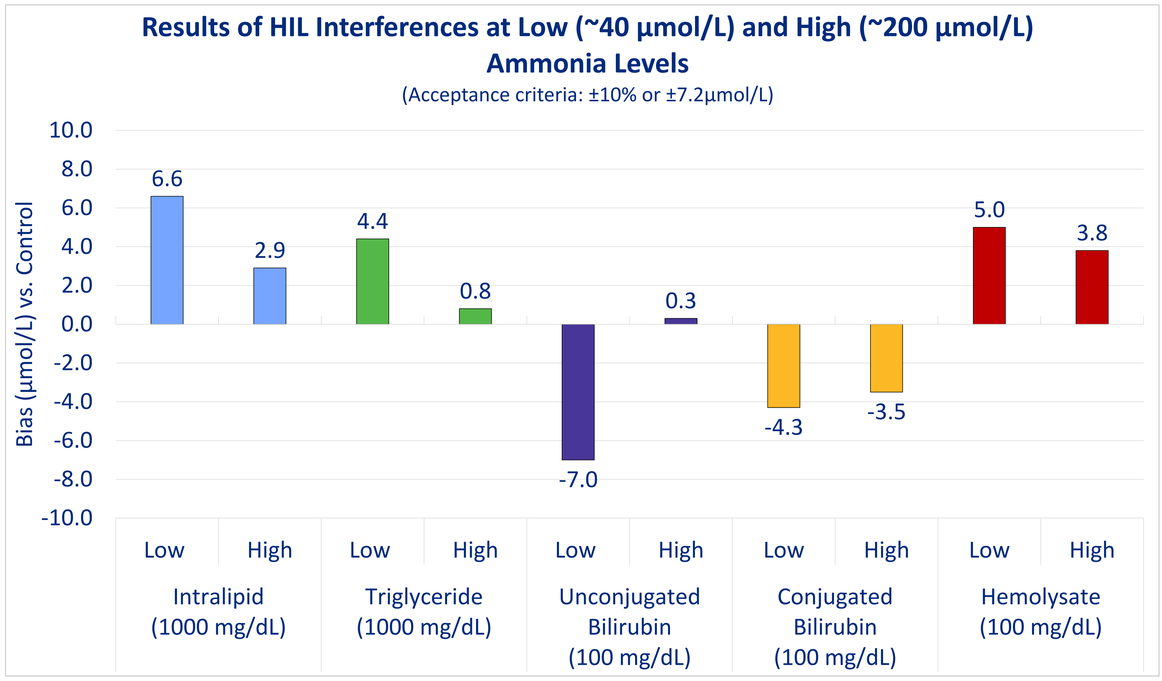
Within-run precision and between run precision (5 days) were evaluated with three levels of commercial QC Bio-Rad Ammonia Controls and Plasma samples.

Table 1. Precision summary

	Mean (µmol/L)	Within-Lab (CV%)	Repeatability (CV%)
Bio-Rad 1 Level 1	52.2	5.7	4.4
Bio-Rad 2 Level 2	121.2	2.9	1.6
Bio-Rad 3 Level 3	284.2	1.5	0.9
Plasma 1	40.6	4.6	3.0
Plasma 2	126	1.8	1.3
Plasma 3	623	1.2	0.9

Interference

Figure 3. Next generation ammonia assay HIL interferences



Summary

Table 2. Summary of Next Generation Ammonia Assay

Sekisui Next Generation Ammonia reagent Performance (DxC 700AU analyzer)	
No Significant Interference from Bilirubin up to	80-100 mg/dL
No Significant Interference from Hemolysis up to	100+ mg/dL
No Significant Interference from Lipemia (Intralipid) up to	1000 mg/dL
No Significant Interference from Lipemia (Triglycerides) up to	1000 mg/dL
Shelf Life	18+ months
Precision (CV%) (Repeatability & Within-Lab)	0.9-4.4%
LoB	2 µmol/L
LoD	6 µmol/L
LoQ	12 µmol/L
Specimen types	LiHep & EDTA
Specimen Bias (n=20 , normal range)	-3.6 µmol/L (Avg)
REACH Compliant	Yes
Analytical Range	12-1017µmol/L

Conclusion

The data demonstrates that Next generation Ammonia Assay is valuable and robust in the accurate assessment of plasma ammonia in clinical chemistry panel.