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Improved assay protocol for measurement of ammonia on the Roche Cobas 8000 automated platform

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A B S T R A C T

Introduction: Ammonia is a metabolite of protein catabolism that, when elevated, may be toxic for tissues, especially for the central nervous system. Elevated ammonia in blood is an indicator and a prognostic factor for hepatic and kidney disease or inherited metabolic disorders in nitrogen metabolism. The accuracy of ammonia determination is influenced by sampling condition, handling, storage and assay itself. Our and other laboratories have been experiencing high frequencies sample error flags while measuring ammonia with glutamate dehydrogenase method on Roche Cobas 8000 platform. To reduce the number of error flags we adapted Roche NH3L protocol by incorporation of an additional onboard routine step for sample pre-dilution.
Material and methods: The AMC NH3L is an adaptation of Roche protocol that uses four fold pre-dilution of the sample in the rerun prior to the analysis. It was assessed for 1.occurrence of absorbance error flags, 2.precision, 3.correlation with Roche method and 4.interference by hemolysis, icterus and lipemia.
Results: The AMC NH3L adaptation demonstrates acceptable within-run and total precision. Comparison studies show no differences between the Roche rerun application and AMC NH3L adaptation. The AMC NH3L adaptation solves 78% of absorbance errors and for samples with high ammonia concentration is less affected by interferences from icterus and hemolysis than the Roche rerun application.
Conclusion: The AMC NH3L adaptation is less prone to instrument error flags and for samples with high ammonia concentration, is more robust to endogenous interferences. The AMC NH3L adaptation is viable alternative to the Roche protocol for the ammonia measurement.

1. Introduction

Increased blood ammonia is an indicator and a prognostic factor for hepatic or kidney disease and inborn errors of amino acid metabolism [1]. Ammonia can be also increased due to therapy with drugs such as valproic acid and chemotherapeutic agents [2,3]. Elevated ammonia is toxic to all organs, especially the brain where it can cause irreversible damage leading to encephalopathy with neurological and cognitive impairment [4]. As both the degree of ammonia elevation and its duration are key determinants of the clinical outcome, hyperammonemia has to be recognized as early as possible. Accurate plasma ammonia measurement remains a challenge as many factors can influence the outcome. Pre-analytical aspects including use of a tourniquet, whether the sample was placed on ice, time of plasma separation and centrifugation temperature play a major role, but a correct analysis with a well-validated and robust method is also crucial [5].

Currently the majority of the clinical laboratories measure ammonia with glutamate dehydrogenase method on the commercially

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available analyzers [6]. Our laboratory has been experiencing high frequencies of sample error flags while using Roche method on the Cobas c502 analyzer. With Roche default application 20.6% of the samples received an error flag for exceeding the absorbance limits on the detector, 2% for icteric and 2% for lipemic sample. The absorbance error flag occurs when the absorbance of the sample exceeds the measuring range of instrument detector and it is independent of the degree of icterus, hemolysis or lipemia.

To solve error flags problem Roche introduced Roche rerun application. Roche rerun is a modification of Roche default application that uses 10 µl sample for the analysis instead of 20 µl that is used in Roche default application. Additionally Roche advised customers to allow the reagent to rest open on the bench for 24 h prior to loading on the instrument. These changes improved method performance but 11.3% of samples still gave absorbance error flag. In order to improve performance of ammonia measurement on Cobas 8000 platform we introduced a modification of Roche protocol, named "AMC (Academic Medical Center) NH3L adaptation" that utilizes in rerun pre-dilution of the sample prior to the analysis. We assessed the AMC NH3L adaptation in terms of susceptibility to the sample error flags, precision, correlation with Roche method and interference from hemolysis, icterus and lipemia. We show that AMC NH3L adaptation represents viable alternative to the Roche protocol for the ammonia measurements on Cobas 8000 automated platform.

2. Material and methods

2.1. Patient samples and reagents

Patient plasma specimens were collected as a part of routine patient care at our institution. Use of these samples for the purposes of method evaluation was classified as a quality assurance program and was granted quality assurance exemption from ethics committee of Amsterdam Medical Center.

EDTA plasma pools was made by pooling several EDTA plasma samples. These EDTA plasma samples were collected in pre-chilled K2 + EDTA Vacutainer tubes (Becton Dickinson), delivered to the lab on ice, separated without delay by centrifugation at 4 °C ± 2 °C and analyzed. Only EDTA plasma samples with low haemolytic (H < 5), icteric (I < 15) and lipemic index (L < 15) were used. Plasma pools were stored at − 80 °C until the analysis.

The Roche NH3L kit (ref. 20766682322), calibrators (ref. 20751995190) and L1(abnormal) and L2 (normal) controls (ref. 20752401 batch number 223930 and ref. 20753009 batch number 223931) were used according to the manufacturer's instruction. Analysis of yearly variation of controls showed that there is little variation between the control batches. The mean coefficient of variation (CV) in 2017 in our laboratory was 2.2% for L1 and 5% for L2 respectively. In this period three different batches of L1 and L2 control were used. Acceptable Water used for analysis was pre-purified with Media Pro120 purification system (ElgaVeolia, UK).

2.2. Assay protocol

Samples were analyzed on Cobas c502 according to the manufacturer's instruction. The only variation in the AMC NH3L adaptation versus Roche protocol was four fold pre-dilution of the sample in the rerun prior to the analysis with Roche default application. In the AMC NH3L adaptation 20 µl of sample was automatically pre-diluted with 60 µl of water and 20 µl of this solution was used for the analysis. The Roche default application on Cobas c502 self was not changed.

### 2.3. Experiment design

2.3.1. Imprecision

Imprecision was tested with 3 replicate analyses in a single run over five consecutive days for two clinically relevant levels of ammonia concentration: L1: Roche control abnormal (ammonia=200 µmol/L) and L2: Roche control normal (ammonia = 60 µmol/L). The precision profile was performed with serial dilution of control material. The point where the CV exceeded 20% was defined as the functional detection limit and served as a means for comparing the methods. Statistical analysis was performed using EP Evaluator (Data Innovations LLC, USA). Precision of AMC NH3L adaptation was considered acceptable if the CV was equal to the Roche rerun method or less.

2.3.2. Correlation

Agreement with Roche method was tested by comparing AMC NH3L adaptation with Roche rerun application. In total 63 samples across the linearity range (21 and 686 µmol/L) were compared. Passing-Bablok regression and Bland-Altman bias analysis were performed using EP Evaluator (Data Innovations LLC, USA). Correlation of AMC NH3L adaptation with the Roche rerun method was considered acceptable if 1) correlation coefficient R was ≤ 0.95, 2) calculated 95% confidence intervals of slope (a) contained 1 and that of intercept (b) contained 0 and 3) bias was ≤ 7.2% (2xCV where CV represents mean CV of L1 and L2 controls measured in our laboratory in 2017).

2.3.3. Interference

Interference studies were conducted using an EDTA plasma pool with low (60 µmol/L) and with high (300 µmol/L) ammonia concentration spiked with intralipid, unconjugated bilirubin or freshly prepared hemolysate. Data were analyzed using GraphPad Prism (GraphPad SoftwareInc., USA). Change of concentration from the base of > 7.2% (2xCV where CV represents mean CV of L1 and L2 controls measured in our laboratory in 2017) was considered significant.

Roche rerun application vs AMC NH3L adaptation
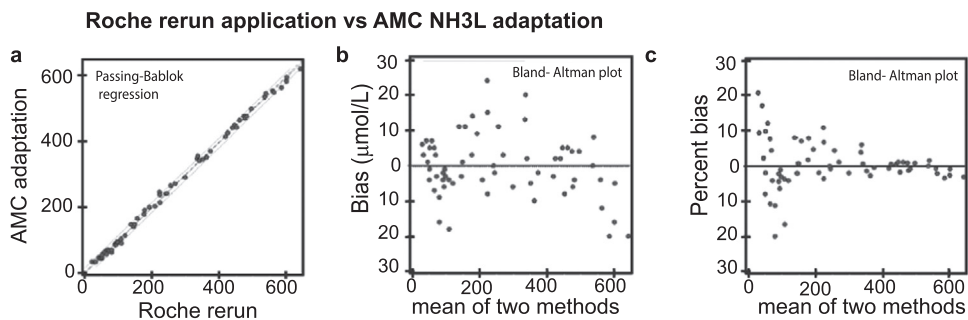
Fig. 1. The AMC NH3L adaptation shows acceptable agreement with the Roche rerun application. Passing-Bablok regression analysis (a) and Bland-Altman plot (b-c) of rerun AMC method relative to Roche rerun method. Regression equation: Y = 0.991 × + 1.4, a: 0.978–1.002, b: −1.2–5.3, R = 0.999, n = 63; % bias = 0.

3. Results

First we tested if AMC NH3L adaptation can solve absorbance errors flags of Roche method. For that samples presenting the absorbance error Roche rerun applications were re-tested with AMC NH3L adaptation. We show that 11 out of 14 samples with absorbance error with Roche protocol did not give absorbance error with AMC NH3L adaptation. This demonstrates that 78% of the absorbance errors is solved with the AMC NH3L adaptation.

Next AMC NH3L adaptation was analyzed in terms of imprecision, correlation with Roche method and interference by hemolysis, icterus and lipemia.

The within-run imprecision for the AMC NH3L adaptation was CV = 1.3% for L1 and CV = 3.1% for L2 and total imprecision was CV = 4.0% for L1 and CV = 8.8% for L2. For Roche rerun application within-run imprecision was CV = 3.1% for L1 and CV = 4.1% for L2 and total imprecision CV = 6.9% for L1 and CV = 10.3% for L2. The precision profile of AMC NH3L adaptation and Roche rerun application demonstrates that for both methods precision increased with increasing ammonia concentration and that for the same ammonia concentration both method showed comparable coefficient of variation. Slight improvement of lower limits of quantification was observed for the AMC adaptation but functional detection limit stayed similar (Supplemental Figure 1). This together shows that AMC NH3L adaptation demonstrates an acceptable precision comparable to that of the Roche rerun application.

In correlation study AMC NH3L adaptation was compared with Roche rerun application. Passing−Bablok regression analysis of AMC NH3L adaptation and Roche rerun application shows acceptable correlation of two methods with correlation coefficient R = 0.999 (Fig. 1 a). Likewise Bland−Altman plot did not show any significant bias between the AMC NH3L adaptation and the Roche rerun (Fig. 1 b-c). These together shows that results found with rerun AMC NH3L adaptation match with the results found with Roche method.

The susceptibility of AMC NH3L adaptation to interferences was compared with that of the Roche method. For samples with low ammonia concentration the AMC NH3L adaptation presented comparable interferences as Roche method (Supplemental Fig. 2). However for high ammonia concentration the AMC NH3L adaptation suffered less from hemolysis, icterus and lipemia and showed no significant interferences up to I index of 1376 (unconjugated bilirubin = 1017 µmol/L), H index of 221 (hemoglobin = 0.260 mmol/L) and L index of 71 (triglyceride = 3.3 mmol/L) (Fig. 2).

4. Discussion

Our laboratory has been experiencing high frequencies of sample error flags while using Roche method on the Cobas c502 analyzer. Previously, two other laboratories have reported similar problems [7,8]. Seiden-Long et al. reduced absorbance error rate by using the Randox reagent as an alternative to the Roche reagent. They demonstrate that Randox reagent shows acceptable
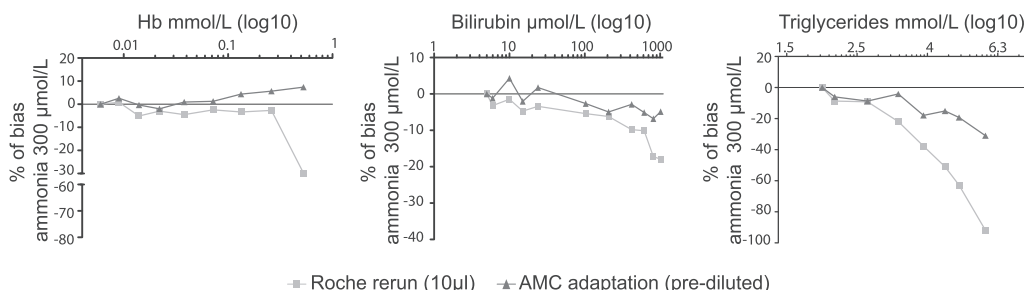
Fig. 2. For high ammonia (300 µmol/L) concentration samples the AMC NH3L adaptation suffers less from interferences from hemolysis, icterus and lipemia. Interference of ammonia measurement in EDTA plasma with high ammonia concentration from hemolysis (left), icterus (middle) and lipemia (right). Results show interference of Roche rerun and AMC NH3L adaptation. Increasing concentrations of interferent are presented on x-axis, and measured bias in comparison to the original result expressed as % of bias on y-axis (n = 2).

precision and accuracy, is less prone to absorbance error flags and is less affected by the interference from hemolysis, icterus and lipemia.

Vassena et al. propose dilution of the Roche R3 reagent. This adjustment reduced absorbance error problems observed with the Roche default application without interfering with the quantification of plasma ammonia as judged from imprecision, correlation and linearity tests.

In our study we present yet another adaptation of the Roche NH3L protocol that solves 78% of the absorbance error flags. This adaptation named "AMC NH3L adaptation" utilizes in rerun four fold pre-dilution of the sample prior to the analysis. We demonstrate that the AMC NH3L adaptation is less prone to the absorbance error flags independently of ammonia concentration in the monster. Moreover for high ammonia concentration samples, it is less susceptible to the interference by hemolysis, icterus and lipemia. At the same time the AMC NH3L adaptation has an acceptable precision and agreement with Roche rerun application. These together evidences that AMC NH3L adaptation is a viable alternative to the Roche rerun application for the ammonia measurement for all specimens showing absorbance error flag.

Acknowledgements

Conflict of interests

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.plabm.2018.e00115.

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