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Transportation and handling of blood samples prior to ammonia measurement in the real life of a large university hospital

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Abstract

Background: Hyperammonemia is neurotoxic and as such can be a medical emergency. Preanalytical factors greatly influence the blood ammonia concentration results. **Aims and methods:** Ammonia concentrations measured in the real life setting of a large hospital after pneumatic transport of blood samples and various time periods before centrifugation were compared to those based on the indications of the reagent manufacturer. In the same routine context, the effects of waiting times of centrifuged samples or after plasma storage at -20°C and -80°C were determined. **Results:** Despite the pneumatic transport, the lead times for sample arrival to the lab were even longer than those recommended for their complete handling until ammonia assay. Ammonia concentration results were not affected by the pneumatic transport of blood samples and by waiting times up to a maximum of 1.75 hours before their centrifugation and 1 hour after centrifugation. Ammonia stability was superior when plasma was stored at -80°C . **Conclusion:** Pneumatic transport and sample handling in the routine practice of our lab do not affect ammonia concentration results provided that waiting times are limited to 1.75 hours before and 1 hour after centrifugation and samples are kept cold. Otherwise, it is better to freeze plasma at -80°C .

Key words: ammonia; pneumatic transport; preanalytical delays; stability.

Authors' participation

FIB: Study concept and design; acquisition of data, analysis and interpretation of data; drafting of the manuscript; statistical analysis, study supervision.

MM, JA, RA, MS, DBR, TP: analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content; statistical analysis.

SD, FM: analysis and interpretation of data, quality managers in the laboratory, critical revision of the manuscript for important intellectual content.

DT, MR, PS: acquisition of data, analysis and interpretation of data, critical revision of the manuscript for important intellectual content.

PEP, SD: ammonia concentration measurements, acquisition of data; technical and material support, statistical analysis.

List of Abbreviations

ACL: Acceptable Change Limit

95%CI: 95% Confidence Interval

EDTA-K2 or -K3: -di or -tri potassium ethylenediaminetetraacetic acid

GGT: gamma-glutamyl-transferase

ALT: alanine aminotransferase

LIS: Laboratory Information System

NCSS: Number Cruncher Statistical Systems

PTS: Pneumatic Tube System

RCPA: Royal College of Pathologists of Australasia

RCV: Reference Change Value

RD: Roche Diagnostics

RD-QC: Roche Diagnostics Quality Control

SFBC: French Society of Clinical Biology

1. Introduction

Ammonia is produced from the breakdown of amino acids and other compounds that contain nitrogen in various cells of the body, and it is toxic at elevated concentrations. In healthy subjects, its transport and metabolism are tightly regulated to maintain low plasma concentrations, and many organs are involved in regulating ammonia homeostasis. Human tissue initially detoxifies ammonia by converting it to glutamine for transport to the liver. Under normal conditions, deamination of glutamine and glutamate in the liver releases free ammonia, which is efficiently converted in the urea cycle to non-toxic urea excreted in urine. The urea cycle is a major mechanism of ammonia removal in humans. Glutamine serves as a major non-toxic storage form and interorgan carrier of free ammonia ions. Most endogenous glutamine is synthesized from glutamate and ammonia in the brain, skeletal muscle and likely in the lungs. In turn, glutamine released into the circulation is catabolized in enterocytes and the kidneys and liver back to ammonia, which is detoxified to urea in the liver [1, 2]. In the physiological state, ammonia concentrations are kept within a narrow range of 10-60 $\mu\text{mol/l}$, depending on the analytical system used for the measurements.

Excess ammonia is neurotoxic and should be measured in patients with unexplained altered mental status. It is now admitted that its toxic effects on the brain play a key role in the pathogenesis of hepatic encephalopathy [3-5]. The etiology of hyperammonemia is usually associated with liver cirrhosis or hepatitis, portosystemic shunt and gastrointestinal bleeding. It can also result from inherited disorders of metabolism such as urea cycle defect or from high-dose chemotherapy [6, 7] or drugs such as valproic acid, heavy exercise and smoking [3, 8].

Measurements of blood ammonia concentrations are greatly influenced by preanalytical factors that may lead to increased values. Special attention is required during

the sampling, the transport of the blood specimens to the laboratory, the storage conditions and the lead time before ammonia assay. A free-flowing venous blood sample should be collected on EDTA as the anticoagulant instead of sodium heparin or sodium fluoride/potassium oxalate in order to reduce non-specific sample-buffer interactions [9-13].

Ammonia concentrations increase spontaneously in blood and plasma after collection. This increase occurs mainly through the release of ammonia from deamination of plasma and cellular proteins and a possible hemolysis. A correlation of ammonia concentration was observed with erythrocyte and platelet counts as well as with plasma activities of γ -glutamyl-transferase (GGT) and alanine aminotransferase (ALT) [12, 14-16]. By contrast, a significant relationship between ammonia concentration and the GGT activity was not observed by Szöke [17]. To slow down in vitro ammonia production, the sample has to be placed immediately on ice after blood collection and analyzed as soon as possible [14-17]. For this reason, blood sample must be quickly transported to the laboratory, within 15 minutes of collection, and immediately centrifuged at +4°C [16, 18]. However, blood collection sites are usually located some distance from the laboratory and rapid separation of plasma from cells is not possible. In large hospital centers, a pneumatic tube system (PTS) is now often used to provide a quick specimen delivery to the laboratory. Nonetheless such a transport system can affect the blood sample's integrity. To our knowledge, no study on the influence of the PTS on the quality of the samples intended for blood ammonia assay has been reported in the literature.

Finally, the biological interpretation of plasma ammonia levels remains sometimes difficult. Despite many works devoted to preanalytical factors and recommendations, little is actually known on the biological variability of ammonia concentrations in healthy subjects. Intra-individual biological variability is available for various analyses but has not yet been

definitively determined for blood ammonia. Consequently, the Reference Change Value (RCV), which distinguishes increased plasma ammonia values raised by the disease from random variations, cannot be calculated for the moment [19-21]. Preliminary results were published by Ucar et al. in 2016 [22].

The objective of our study was to assess the influence of the PTS and of the management times of blood samples, potentially fragilized after pneumatic transport to the lab, on ammonia concentration results in a large university hospital through the use of two protocols [23]. In the first protocol, ammonia concentration results, obtained in a real world hospital practice setting were compared to those obtained using “ideal” conditions as described by the manufacturer [24]. In the second protocol, the ammonia concentrations measured using the normal process of the laboratory were compared with those obtained in dysfunctional situations with increased delays. Also, in case of a major technical problem or in a study protocol, results were compared after one week of plasma storage at -20°C and -80°C.

2- Materials and methods

2-1 Blood sampling intended for ammonia analysis and the conditions of transport to the laboratory

The manufacturer, Roche Diagnostics (RD), recommends collecting the blood in tubes with EDTA-K2 from stasis-free veins in fasting patients. The analysis has to be performed within 20 to 30 minutes of venipuncture, with tubes placed immediately on ice and quickly centrifuged at +4°C (24).

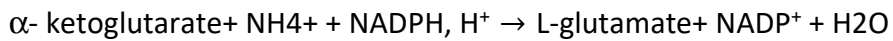
In the Pitié-Salpêtrière Hospital medical units, the blood sample is collected by a nurse in EDTA-K3 blood tubes (Becton Dickinson Vacutainer® 3 mL, EDTA-K3 5.4 mg, reference 368857, 94150 Rungis, France), gently homogenized and quickly enveloped in a synthetic ice device, Carbhypad® (Carboservice eutectic gel reference SAP 10544458, +2°C - +8°C). It is then placed with the Carbhypad® in a bag, which is then placed in a cartridge and transported by PTS to the lab. After the blood sample arrives at the lab, the ammonia analysis request is registered in the LIS; thereafter, the sample is hand delivered to a technician who immediately starts its centrifugation at +4°C for 10 minutes at 1500 x g (Eppendorf 5810 Bench Top Centrifuge, Hamburg, Germany) before performing the ammonia assay.

The PTS (Aerocom®, Germany) is an underground pneumatic transport system using 160-mm diameter cylindrical transport cartridges for biological samples. The cartridges are sent to the laboratory from several stations located in different medical service points within Pitié-Salpêtrière Hospital. The length of the line used in the study is 246 meters. There are three switches and five curvatures before reaching a coupler. The average transfer time to the reception center for the biological samples is 4 to 5 minutes, i.e. 49 to 61 meters per minute or about 1 meter per second. After arrival at the reception center, the cartridges are then inserted in a transport cylinder and sent to the biochemistry laboratory, located two floors below.

2.2 Ammonia measurement

The ammonia assay was performed on a Modular® P800 analyzer (Roche Diagnostics, Basel, Switzerland) according to the enzymatic method described by van Anken and

Schiphorst [25] using the manufacturer's reagent kits [26]. The enzymatic reaction was catalyzed by glutamate dehydrogenase (GLDH) with NADPH as coenzyme and presented below [25, 26].



The decreased absorbance was measured at 340 nm [24 -26]. The measuring range was from 5.87 up to 587 $\mu\text{mol/l}$. Plasma hemolysis, icterus and lipemia were assessed on the Modular® P800 as H, I and L indices, respectively. Up to a hemolysis index (HI) of 50 (an approximate hemoglobin concentration of 0.50 g/l), it has been shown there is no significant interference. For HI>50, ammonia concentration measurements were automatically invalidated [24].

At the time of the study, ammonia intra-assay precision was verified on low level RD Quality Control specimens (reference 20752401) and also on two plasma pools, no. 1 and no. 2, in order to take into account the matrix effect of the human blood sample. Intra-laboratory inter-assay precision was assessed from the results of RD Quality Controls at low and medium levels (references 20752401 and 20753009, respectively) collected over a 6-month period, and from those from a third plasma pool (no. 3), collected twice per day over a 2-week period, for the same reason as presented above. Plasma pools no. 1, 2 and 3 were prepared with the remaining volumes of the supernatant of EDTA-3K blood samples already analyzed for various patients.

The ammonia measurements in both parts of protocol 2 (protocols 2-1 and 2-2) described below, were performed in duplicate and biases were calculated.

2.3 Patients and specimens

Flow charts of the sample analysis pathways in protocols 1 and 2 are presented Figure 1.

Protocol 1 - (Figure 1A). Effects on ammonia concentration results, of pneumatic tube system (PTS) and of elapsed time between blood sample collection and assay.

Ammonia results were compared when blood samples were processed according to the routine hospital practice, including the PTS transport, versus the conditions described by the manufacturer [24]. The lead times were measured before blood sample arrival to the lab, before their registration in the Laboratory Information System (LIS), before their centrifugation and before the ammonia assay.

Twenty blood samples were collected from 15 patients hospitalized in an intensive care unit of the Hepatology Department in accordance with the medical prescription for ammonia analysis. Blood was collected twice in four patients and three times in one patient on different days of hospitalization. For each subject the blood was collected into three tubes (TA, TB, TC) with EDTA anticoagulant. Contrary to recommendations, the patients were not fasting.

One tube (TC) was used for the requested ammonia assay. The two additional tubes (TA and TB) were used for improved management of samples intended for ammonia concentration measurement and consequently the quality of the results. This step was important in the context of the obligatory accreditation process for the laboratory, which guarantees the reliability of the medical biology examinations carried out and the quality of the medical service [27]. For a comparison of real life blood handling with direct application for routine in vitro diagnostic purposes, the consent of the patient was not required for the study.

Tubes TA and TB, enveloped in the Carbhypad[®], were transported by foot immediately after the blood collection to the laboratory in less than 10 minutes. Once at the lab, TA was immediately centrifuged at +4°C for 10 minutes at 1500 x g. The TA ammonia

assay was performed as soon as possible on the Modular[®] P800 analyzer. The results obtained on TA samples became the referent values for protocol 1. Tube TB was left enveloped in the Carbhypad[®] without centrifugation until the arrival of tube TC, which had been carried by PTS to the laboratory. TB was the control for the effects of the blood sample waiting time at +4°C before centrifugation on ammonia results. Once the bag containing the tube TC, transported by PTS, arrived at the lab and ammonia analysis had been registered, TC was associated with TB and both were given in person to the technician for their centrifugation at +4°C for 10 minutes at 1500 g and for ammonia assay. TC was the control for the effects of PTS on the ammonia results.

Protocol 2 (protocols 2-1 and 2-2) (Figure 1B).

The blood samples were issued from patients in different clinical care settings of the hospital with a request for ammonia concentration measurement. Only the results of the first assay were returned to the physician.

The concentrations measured on centrifuged samples in the normal conditions of the lab were compared with those obtained in dysfunctional situations causing increased delays before ammonia assay (labeled protocol 2-1). Ammonia concentrations measured were also compared after one week of plasma storage at -20°C and at -80°C (labeled protocol 2-2).

Protocol 2-1: Effects on ammonia concentration results, of the different waiting times at +4°C of the centrifuged primary tubes and of the storage at +4°C of separated plasma.

After a first ammonia measurement, the assays were performed again on the remaining plasma supernatant from the same primary centrifuged tube, and then recapped

and stored for different times at +4°C. The first ammonia concentration measurement became the referent in protocol 2-1 and was identified as Primary Tube 0 concentration (PT0). After the PT0 measurement, the same primary tube was stored for one more hour at +4°C before the second ammonia assay, the result of which was called PT1. After the PT1 measurement, the primary tube was kept for three more hours at +4°C (i.e. 4 hours after PT0 measurement) before the third ammonia assay, the result of which was called PT3. After the PT1 measurement, one aliquot of plasma supernatant was also separated from the primary tube and was left for 3 hours at +4°C before the ammonia assay, the result of which was called AL3.

Protocol 2-2: Ammonia concentration results after plasma storage for one week at -20°C and -80°C.

The primary tube stored for one hour at +4°C with ammonia concentration PT1 was also used to prepare two plasma aliquots of 250 µL each. The two aliquots were stored for one week at -20°C and -80°C, respectively. PT1 was the referent ammonia concentration in protocol 2-2.

2.4 Data and Statistical analysis

For the precision criteria of the ammonia assay, the obtained results were compared with the specifications on allowable analytical error as defined by the French Society of Clinical Biology (SFBC) from the state-of-the-art data. For the assays performed in duplicate in protocol 2, the biases were calculated as %bias= 100*(value at specific condition-reference value)/reference value).

Statistical significance between groups for ammonia concentration in the different protocols was determined by the paired Student's t-test using Number Cruncher Statistical Systems 200X software (NCSST, Kaysville, UT, USA). A p-value less than 0.050 was considered significant.

The mean differences between the referent ammonia concentrations and those measured on the same blood samples handled according to various preanalytical conditions were calculated in the different protocols. The mean percentage deviations (biases) were calculated as well and were compared with the Acceptable Change Limit (%ACL) [30, 31]. ACL is used for interpreting a measured difference based on the analytical imprecision (coefficient of variation [CV]). %ACL is equal to $1.96 \sqrt{2} CV_a$, wherein $1.96\sqrt{2}$ corresponds to the standard deviation for the bidirectional probability of change fixed at 95%, and CV_a to the inter-assay imprecision calculated from low level RD Quality Control results collected over 6 months before the study [28, 30- 32].

Comparative studies were also performed using PC Valtec 1997 software for the Passing-Bablok linear regression equation [33]. The comparison requirements are based on specifications defined by the SFBC from the state-of-the-art data at three ammonia concentrations (50, 100 and 200 $\mu\text{mol/l}$), with tracking standards in absolute value expressed in $\mu\text{mol/l}$ of 15, 25 and 42, respectively, and interpretation standards of 7, 13, 25, respectively. [28]. Boxplots were plotted using GraphPad Prism (version 1.7).

Finally, the results were also compared with the Royal College of Pathologists of Australasia (RCPA) criteria (i.e. $\pm 5 \mu\text{mol/l}$ for ammonia concentrations $\leq 50 \mu\text{mol/l}$, and $\pm 20\%$ when up to 50 $\mu\text{mol/l}$) [28, 29].

3 Results

3.1 Precision criteria of ammonia assay on Roche Diagnostics P800 Modular[®] analyzer

at the time of the study.

The results of mean \pm standard deviation (SD) of the ammonia concentrations measured in the lab and expressed in $\mu\text{mol/l}$, are indicated below.

On Roche Diagnostics Quality Controls (RD-QC):

Intra-assay precision - low level RD-QC: 60.2 ± 3.0 (n=30),

Inter-assay precision - low level RD-QC: 42.6 ± 3.1 (n=29),

- medium level RD-QC: 224.6 ± 6.7 (n=30).

On plasma pools:

Intra-assay precision - plasma pool no.1: 51.3 ± 5.3 (n=30),

- plasma pool no.2: 338.7 ± 2.9 (n=29),

Inter- assay precision - plasma pool no.3: 107.6 ± 3.7 (n=29).

The coefficients of variation (%CV) were always inferior to 5.0 except for the intra-assay precision on plasma pool n°1 in which it was equal to 10.3.

%ACL was equal to 20.5 and 9.6 for low and medium level RD ammonia controls, respectively.

For the ammonia assays performed in duplicate (Protocol 2), the calculated mean biases for PT1 (n=36), PT3 (n=34) and AL3 (n=37) were +3.0%, +2.1% and +3.1%, respectively. After plasma storage at -20°C (n= 31) and at -80°C (n=28), they were -1.1% and +2.4%, respectively. These results were in accordance with the SFBC criteria for a range of values from 10 to 240 $\mu\text{mol/l}$.

3.2 Effects on ammonia concentration results, of the pneumatic tube system (PTS) and of elapsed time between blood collection and assay (Protocol 1).

The median times for tubes TA, TB, TC from zero time of blood collection (T0) to the various preanalytical steps are presented in Table 1 (n=20). For referent TA, the time period from blood collection to ammonia measurement recommended by the manufacturer (less than 30 minutes) was observed with the exception of one specimen (35 minutes). In contrast, for TB and TC tubes, which were handled together after the arrival of TC at the lab, the median time period was longer, or 74 minutes. The median time before tubes centrifugation was 58 minutes.

The ammonia concentration results of TA, TB, TC are presented in Table 2 and boxplots in Figure 2. The mean ammonia concentrations were close to the normal threshold in the laboratory ($\leq 50 \mu\text{mol/l}$). The results of TB versus TA are not significantly different as confirmed by the paired t-test ($p= 0.130$). However, they are statistically different for TC carried by PTS versus TB ($p=0.039$) and for TC versus TA ($p=0.003$).

The bias between the results of TC versus TA is above that of TC versus TB and of TB versus TA, but it is still below the ACL of 20.5% determined on low level RD Quality Control with ammonia concentration $<50 \mu\text{mol/l}$.

Comparative studies according to Passing-Bablok linear regression are accepted in accordance with the SFBC specifications for TB versus TA, TC versus TA and also TC versus TB at the three levels of ammonia concentration, 50, 100, and 200 $\mu\text{mol/l}$ [28].

3.3 Effects on ammonia concentration results, of different waiting times at +4°C of the centrifuged primary tubes and of the storage at +4°C of separated plasma (protocol 2-1).

In protocol 2-1, it was possible to gather PT1, PT3, AL3 ammonia results for only 41 primary tubes out of 49. Actually, for some specimens it has been difficult to manage the right waiting time before PT1, PT3, AL3 ammonia tests (protocol2-1) or to have a sufficient volume of supernatant for PT3 and AL3 assays after the preparation of plasma aliquots (protocol 2-2).

The results are presented in Table 3 and boxplots in the Figure 3A (n=41). PT1 concentrations are named PT12-1. PT0 are the referent ammonia concentrations in protocol 2-2.

The mean ammonia concentrations increase in relation to the time storage of the primary centrifuged tubes and compared with PT0 at +4°C. Significant paired t-tests ($p < 0.001$) were calculated between the results of PT12-1 versus PT0, PT3 versus PT0, and AL3 versus PT0.

The mean bias between the PT12-1 and PT0 results is inferior to the %ACL determined on low level RD Quality Control. This is not the case for the PT3 versus PT0 results or the AL3 versus PT0 results.

Comparison studies of PT12-1 versus PT0, PT3 versus PT0 and AL3 versus PT0 according to Passing-Bablok linear regression are satisfactory only for PT12-1 versus PT0 results [28].

RCPA specifications were verified for the difference between PT12-1 and PT0 results only [29].

3.4 Ammonia concentration results after plasma storage for one week at -20°C and -80°C (protocol 2-2).

The results are presented in Table 3 and boxplots in Figure 3B. PT1 concentrations were measured on 49 specimens and are named PT12-2. They are the referent ammonia concentrations in protocol 2-2.

Two outliers were starred by the Number Cruncher Statistical Systems software i.e. rows separated from the rest of the data in which the R of Student is >2 . These outliers were eliminated in the analysis of the differences between the results ($n=47$). Compared to PT12-2, the mean of differences and the 95%CI in the results were higher when plasma samples were stored at -20°C than at -80°C (8.3 ± 8.3 ; 95% CI of 5.9 to 10.7 and 2.3 ± 6.5 ; 95% CI of 0.4 to 4.2, respectively) for the range of values from 12 to 225 $\mu\text{mol/l}$.

The comparative studies of Passing-Bablok were conducted with and without the outliers ($n=49$ and $n=47$, respectively). In both cases, the comparative studies were accepted, according to the SFBC specifications, at the three levels of ammonia concentration when plasma samples were kept at -80°C , but they were refused at the low ammonia concentration when the samples were stored at -20°C [28]. When the outliers were deleted, the correlation coefficients improved for the two storage temperatures of -20°C and -80°C .

4 Discussion

The same ammonia assay is performed on most automated systems, which should provide harmonious results between laboratories [34]. However, a few preanalytical factors could affect the reliability of the results [9-18]. It is now established that the blood sample must be collected on EDTA as anticoagulant and placed immediately on ice in order to stop the cellular metabolism and to limit the activity of the enzymes producing in vitro ammonia. The time between the blood collection and the ammonia assay must be as short as possible, at best less or equal to 30 minutes. Nevertheless, the recommended time for transport and

handling of blood sample before ammonia assay is hardly observed in current practice because the lab is often located at a distance from the blood collection point and/or has a large flow of biological samples.

In this study, we have assessed the effects on ammonia results of blood sample pneumatic transport, and subsequently, of their handling times in the routine practice of a large university hospital laboratory (protocol 1) and in the case of a slowed down process with increased delays (protocol 2). The study was realized on fresh blood samples from hospitalized patients.

The correlation between plasma ammonia concentrations and gamma-glutamyl transferase (GGT) activity, alanine aminotransferase (ALT) activity and red blood cells (RBC) and platelet counts was not studied [12, 15, 17, 35].

First of all, we have assessed the effects on ammonia concentration results, of pneumatic tube transport (PTS) and of elapsed time before blood sample centrifugation and assay (TA, referent tube, carried by foot and handled as specified by the reagent manufacturer and literature data; TB carried by foot with TA and waiting for the TC arrival at the lab; TC transported by PTS)- Protocol 1, Table 1, Figure 1.

The impact of PTS on the integrity of blood samples intended for ammonia concentration measurement was assessed by the comparison of ammonia concentrations of TC versus TA and TC versus TB.

The influence of the waiting time on the no centrifuged blood sample was assessed by the comparison of ammonia concentrations of TB with those of TA.

It was observed that the median time for arrival of tubes TA and TB, both carried by foot to the lab, was less than 5 minutes, with a maximum time of 8 minutes thanks to the geographical proximity of the clinical unit and the exceptional conditions for their transportation by special carrier. The median time period from the blood collection to ammonia measurement of tube TA was inferior to the maximum time indicated by the manufacturer of ammonia reagents.

The median time for the arrival of tube TC to the lab, after PTS transport, was 35 minutes, with a maximum time of 108 minutes because of a one-time pneumatic system malfunction. A median time of 58 minutes (mean \pm SD equal to 70.2 ± 36.3 min) was needed before TB and TC centrifugation, and finally a median time of 74 minutes before TB and TC ammonia assay (mean time \pm SD equal to 83.3 ± 41.2 min). Anyway, the minimum time period of 45 minutes observed for the complete treatment before ammonia analysis of tube TC transported by PTS and of tube TB transported by foot was higher than the 30 minutes recommended by the manufacturer [24].

The mean differences in ammonia concentrations between TB versus TA, TC versus TA and TC versus TB are very close, in particular for TC versus TB and TB versus TA. It has to be noted that the only difference between TB and TC resides in the means of blood sample transportation (protocol 1 Table 2, boxplots in Figure 1).

The paired t-test highlights that there is no significant difference for the results of tube TB (transported on foot and with a wait time before centrifugation) versus referent TA ($p=0.130$), whereas differences were significant for TC transported by PTS versus TB transported on foot ($p=0.039$) and highly significant for TC versus TA ($p=0.003$).

These differences in ammonia results certainly exist between tubes TA, TB and TC, handled in different preanalytical conditions, but they have to be compared with the analytical specifications defined by the SFBC from the state-of-the-art data for the ammonia measurement. As such, tolerable or not tolerable analytical variability at different ammonia concentrations can be evaluated, taking into account the reliability of the assay and standards of interpretation. The results obtained are in accordance with tracking and interpretation standards in absolute values of the SFBC. This is confirmed by the comparative studies of the TC versus TA and TC versus TB results according to the Passing-Bablok linear regressions, which are accepted at the three levels of ammonia concentration, including 50 $\mu\text{mol/l}$.

The differences in the results for TB versus TA, TC versus TB and TC versus TA are also in accordance with RCPA specifications.

In conclusion, the transport of samples by PTS (TC) and a delay up to 1.75 hours (mean \pm SD in minutes: 70.20 \pm 36.30) before their centrifugation (TB and TC), in comparison with the referent tube (TA), had no significant effects on the ammonia concentration results. However, this study was limited by a low number of TA, TB, TC triplicates (n=20) and by the low ammonia concentrations ($\leq 50 \mu\text{mol/l}$) for 12 triplicates. Follow-on studies would benefit from the inclusion of more blood samples from patients with hyperammonemia. The literature recommends a very short elapsed time between blood collection and centrifugation, but this means that in “real life”, blood samples must be centrifuged in the clinical care unit [12,15, 36- 38].

In addition we have studied the effects on ammonia concentration results, of different waiting times at +4°C of the centrifuged primary tubes and of the storage of

separated plasma [(PT0 - first assay, PT12-1 - second assay (PT0 + one hour at +4°C), PT3 - third assay (PT12-1 + 3 hours at +4°C), AL3 ammonia concentration determined on separated plasma after PT12-1 measurement and stored 3 hours at +4°C (n=41)- protocol 2-1, Table 3, boxplots in Figure 3A).

The ammonia measurement was repeated after the primary tube storage of one hour. The results between the first assay (PT0) and the second assay (PT12-1) were significantly different according to the paired t-test ($p < 0.0001$). In protocol 2-1, as in the protocol 1, a high percentage of ammonia concentrations (67%) were found to be normal ($\leq 50 \mu\text{mol/l}$). Otherwise, for these low ammonia concentrations (n=32), the mean difference \pm SD between the PT12-1 vs. PT0 results was $5.47 \pm 4.05 \mu\text{mol/l}$, which is very close to $5 \mu\text{mol/l}$, as recommended by the RCPA, for ammonia concentrations $\leq 50 \mu\text{mol/l}$ (data not shown). The comparative studies using Passing-Bablok linear regressions were accepted at the three concentration levels. In our study, the acceptable waiting time of one hour before ammonia assay for centrifuged blood samples is close to the literature data for EDTA-K2 plasma samples that are not transported to the lab by PTS [38].

In contrast, it was different for the PT3 results after three more hours of storage of the primary tube after PT12-1 measurement, i.e. four hours after PT0. They were significantly different according to the paired t-test ($p < 0.0001$), with a mean difference \pm SD of $26.2 \pm 11.6 \mu\text{mol/l}$ (mean bias 80%). This increase of in vitro ammonia concentration must be related to the late processing of the sample, the different methods of handling, temperature variations, supernatant evaporation, possible diffusion of components between packed red cells and supernatant, and the deterioration of blood components (14, 16).

When plasma from the primary tube PT12-1 was separated and stored for 3 hours at +4°C (AL3), the mean bias was still high (39%) but was much lower than without the plasma separation (PT3). Nevertheless, it is still superior to the %ACL determined from RD low level control.

The observed difference between PT3 and AL3 results may support the hypothesis for PT3 of a diffusion of components from red blood cells into the plasma supernatant due to the absence of separator gel in the primary tube. Moreover, the tubes were not centrifuged again before the ammonia assays.

The comparative studies of the results of PT3 versus PT0 and AL3 versus PT0 according to Passing-Bablok linear regression were refused at the different ammonia concentration levels of 50, 100, 200 µmol/l.

In conclusion, these results are not affected by a one-hour delay after blood sample centrifugation and prior to ammonia concentration measurement. Moreover, it must be underlined that the blood samples were carried by PTS to the laboratory and had a median wait time of 58 minutes (mean wait time 70.20 ± 36.3 minutes) before their centrifugation, in reference to the protocol 1 results. Such findings, after addition of the mean lead times prior to ammonia assay, do not totally corroborate those of Hashim [39] who stated that the elapsed time from blood collection to result completion has to be inferior to 120 minutes.

Finally, in protocol 2-2, Table 4, boxplots in Figure 3B (n=49), the **ammonia concentrations measured after plasma storage for one week at -20°C and -80°C**, confirmed the better stability of the plasma ammonia after one week of storage at -80°C, which is consistent with which has already been reported in the literature [16]. Moreover, in our

study, the plasma freezing was performed after a one-hour waiting time at +4°C of the centrifuged primary tube.

Gifford et al. developed a simple method to stabilize specimens intended to ammonia analysis for more than 12H post blood collection. This method consists in the pretreatment of plasma specimens with enzyme inhibitors sodium borate/L-serine combined with transport and storage at +4°C. After such a pretreatment, they have shown that the rate of in vitro ammonia generation was inferior to 0.5 $\mu\text{mol}/\text{L}/\text{h}$ for samples with both normal and increased ALT and/or GGT activity. They have also confirmed that storing specimens at -80°C can preserve the ammonia stability independently of the activity of ALT and/or GGT, nevertheless specimens have to be thawed in a 30 minutes time precisely [16].

In our case the hospital group does not receive blood samples from remote sites requiring a long transport time for samples. The ammonia test requests are mainly issued from 2 hospitals on 3 which are geographically close. The two hospitals are connected to the laboratory by a PTS for a short time transport of samples. It was clear that there was an urgent need to assess the effect of this mean of transport on the quality of fresh blood samples intended to ammonia measurement which was not reported previously. On the other hand, in real life of the lab, the time for a complete treatment of blood samples before ammonia assay is longer than that recommended. The waiting times can alter the stability of the sample, especially after a pneumatic transport.

Following our study, in order to further reduce the delay between the blood collection and the sample centrifugation, it was decided that after blood collection the sample would be placed enveloped in Carbhypad in a black bag for the pneumatic transport. The black bag means that the sample analysis must be carried out in extreme urgency. Once arrived at the

lab, and at the exit of the PTS, the black bag is easily identified among all the others and thus handled quickly.

Concerning the centrifuged samples, beyond a waiting time of one hour before ammonia assay the supernatant must be frozen at -80°C .

5. Conclusion

After the study performed in the real life setting of the university-affiliated Pitié-Salpêtrière-Charles Foix Hospital Group, we can conclude that the pneumatic transport plus the laboratory handling times of fresh blood samples intended for ammonia concentration measurement do not have significant effects on the results. Ammonia concentrations are not affected by a waiting time of up to 1.75 hour (i.e. 105 minutes) before and 1 hour after centrifugation, provided samples are kept cold. Otherwise, it is better to freeze plasma samples at -80°C .

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REFERENCES

- [1] Holecek M. Evidence of a vicious cycle in glutamine synthesis and breakdown in pathogenesis of hepatic encephalopathy- therapeutic perspectives. *Metab. Brain. Dis.* 29 (1) (2014) 9-17.
- [2] Wright G, Noiret L, Olde Damink SWM, Jalan R. Interorgan ammonia metabolism in liver failure: the basis of current and future therapies. *Liver International.* 31 (2) (2011) 163-175.
- [3] A. Auron, P.D. Brophy. Hyperammonemia in review: pathophysiology, diagnosis, and treatment. *Pediatr. Nephrol.* 27 (2) (2012) 207-222.
- [4] F. Gundling, E. Zelihic, H. Seidi, B. Haller, A. Umgelter, W. Schepp, C Dodt. How to diagnose hepatic encephalopathy in the emergency department. *Ann. Hepatol.* 12 (1) (2013) 108-114.
- [5] P.J. Parekh, L.A. Balart. Ammonia and its role in the pathogenesis of hepatic encephalopathy. *Clin. Liver. Dis.* 19 (3) (2015) 529-537.
- [6] Yi HJ, Hong KS, Moon N, Chung SS, RA L, Kim KH. Acute hyperammonemic encephalopathy after 5-fluorouracil based chemotherapy. *Ann. Surg. Treat. Res.* 90 (3) (2016) 179-182.
- [7] Nott L, Price TJ, Pittman K, Patterson K, Fletcher J. Hyperammonemia encephalopathy: an important cause of neurological deterioration following chemotherapy. *Leuk. Lymphoma.* 48 (9) (2007) 1702- 1711.
- [8] Lewis C, Deshpande A, Tesar GE, Dale R. Valproate-induced hyperammonemic encephalopathy: a brief review. *Curr. Med. Res. Opin.* 28 (6) (2012) 1039-1042.

- [9] Goldstein BN, Wesler J, Nowacki AS, Reineks E, Natowicz MR. Investigations of Blood ammonia analysis: test matrices, storage, and stability. *Clin. Biochem.* 50 (9) (2017) 537-539.
- [10] F. da Fonseca-Wollheim; M. Van Dam. Interference by heparin in enzyme determination of plasma ammonia depends on reagent composition. *Clin. Chem.* 38(9) (1992) 1921-1922.
- [11] N. Wannasilp, K. Sribhen, N. Pussara, N. Opartkiatikul. EDTA should be the anticoagulant of choice for the measurement of plasma ammoniemia: report of problem sample. *Clin. Chim. Acta.* 357 (1) (2005) 84-85.
- [12] N. Nikolac, J. Omazic, A.M. Simundic. The evidence based practice for optimal sample quality for ammonia measurement. *Clin. Biochem.* 47 (12) (2014) 991-995.
- [13] L. Dukic, A.M. Simundic. Short-term and long-term storage stability of heparin plasma ammonia. *J. Clin. Pathol.* 68 (4) (2015) 288-291.
- [14] F. da Fonseca-Wollheim. Preanalytical increase of ammonia in blood specimens from healthy subjects. *Clin. Chem.* 36 (8 Pt 1) (1990) 1483-1487.
- [15] P. Schuff- Werner, M. Steiner. Preanalytical ammonia generation: a race with time but not with temperature. *J. Clin. Pathol.* 68 (2015) 757-758.
- [16] J.L. Gifford, W.N.T. Nguyen, L. de Koning, I. Seiden-Long. Stabilizing specimens for routine ammonia testing in the clinical laboratory. *Clin. Chim. Acta.* 478 (2018) 37-43.
- [17] D. Szöke, S. Birindelli, S. Pasqualetti, A. Dolci, M. Panteghini. Are blood ammonia concentrations dependent on gamma- glutamyl- transpeptidase levels in plasma? *J. Clin. Pathol.* 69 (6) (2016) 551-552.

- [18] J.H. Howanitz, P.J. Howanitz, C.A. Skrodzki, J.A. Iwanski. Influences of specimen processing and storage conditions on results for plasma ammonia. *Clin. Chem.* 30 (6) (1984) 906-908.
- [19] C.G. Fraser. Inherent biological variation and reference values. *Clin. Chem. Lab. Med.* 42 (7) (2004) 758-764.
- [20] C. Ricos, F. Cava, J.V. Garcia- Lario, A. Hernández, N. Iglesias, C.V. Jiménez, J. Minchinela, C. Perich, M. Simon, M.V. Domenech, V. Alvarez. The reference change value: a proposal to interpret laboratory reports in serial testing based on biological variation. *Scand. J. Clin. Lab. Med.* 64 (3) (2004) 175-184.
- [21] C. Ricos, V. Alvarez, F. Cava et al. Biological variation database and quality specifications for imprecision, bias and total error. The 2006 update. Available at: <http://www.westgard.com/guest32/htm>.
- [22] F. Ucar, G. Erden, S. Ozdemir, N. Ozcan, E. Bulut, A. Ozturk. First data on the biological variation and quality specifications for plasma ammonia concentrations in healthy subjects. *Clin. Chem. Lab. Med.* 54 (5) (2016) 857-863.
- [23] G.V. Kapoula, P.I. Kontou, P.G. Bagos. The impact of pneumatic tube system on routine laboratory parameters: a systematic review and meta-analysis. *Clin. Chem. Lab. Med.* 55 (12) (2017) 1834-1844.
- [24] Roche Diagnostics Ammonia (NH₃) Method sheet/ Package Insert. Ammonia information reference 11877984. Analyzer Modular P (2017).
- [25] H.C. van Anken, M.E. Schiphorst. A kinetic determination of ammonia in plasma. *Clin. Chim. Acta.* 56 (2) (1974) 151-157.

- [26] F. da Fonseca-Wollheim; K.G. Heinze. Which is the appropriate coenzyme for the measurement of ammonia with glutamate dehydrogenase. *Eur. J. Clin. Chem. Clin. Biochem.* 30 (9) (1992) 537-540.
- [27] M. Plebani, L. Sciacocelli, A. Alta. Quality indicators for the total process testing. *Clin. Lab. Med.* 37 (1) (2017) 187-205.
- [28] A. Vassault, D Grafmeyer, J de Graeve, R Cohen, A Beaudonnet, J Bienvenu. Quality specifications and allowable standards for validation of methods used in clinical chemistry. *Ann. Biol. Clin.* 57 (6) (1999) 685-695.
- [29] Royal College of Pathologists of Australasia allowable limits of performance. Available at : <https://www.westgard.com/rcpa.htm#alcohol>, Accessed February, 2020.
- [30] C. Giroud, M. Dumontet, A. Vassault, E. Braconnier, G. Ferard. Recommendations for expressing uncertainty of measurement of quantitative results in laboratory medicine. *Ann. Biol. Clin.* 65 (2) (2007) 185-200.
- [31] G.H White and I. Farrance. Uncertainty of measurement on quantitative medical testing. A laboratory implementation guide. *Clin. Biochem. Rev.* 25 (4)(2004): S1-S24.
- [32] C. Oddoze, E. Lombard, H. Portugal. Stability study of 81 analytes in human whole blood, in serum and in plasma. *Clin. Biochem.* 45 (6) (2012) 464-469.
- [33] Passing H, Bablok W. A new biometrical procedure for testing the equality of measurements from two different analytical methods. Application of linear regression procedures for method comparison studies in clinical chemistry, Part 1. *Clin. Chem. Clin. Biochem.* 21 (11) (1983) 709-720.

- [34] C. Markus, M. Metz. Comparison of plasma ammonia results from seven different automated platforms in use throughout Australia. *Clin. Biochem.* 50 (6) (2017) 331-335.
- [35] F. da Fonseca-Wohlleim. Deamidation of glutamine by increased plasma gamma-glutamyl transferase in a source of rapid formation in blood and plasma specimens. *Clin.Chem.* 36 (8) (1990) 1479-82.
- [36] B. Maranda, J. Cousineau, P. Allard, M. Lambert. False positives in plasma ammonia measurement and their clinical impact in a pediatric population. *Clin. Biochem.* 40 (8) (2007) 531-535.
- [37] J. Favresse, N. Despas, D. Gruson, M.F. Vincent, C. Fillée. Pre-analytics of ammonia: Crioplast® devices as an alternative to icy water. *Clin. Chim. Acta.* 472 (2017) 102-103.
- [38] J. Favresse, N. Despas, L. Boland, D. Gruson, P.F. Laterre, M.A. Nassogne, M.F. Vincent, C. Fillée. Preanalytics of ammonia: stability, transport and temperature of centrifugation. *Clin. Chem. Lab. Med.* 56 (3) (2018) e65-e68.
- [39] I.A. Hashim, J.A. Cuthbert. Elevated ammonia concentrations: Potential for pre-analytical and analytical contributing factors. *Clinical Biochemistry* 47 (2014) 233-236.

Legends of the Tables and of the Figures

Tables

Table 1: Median time periods, expressed in minutes, between blood collection and the different steps of sample treatment in the lab until ammonia assay [(TA; TB; TC) n=20]. *TA: referent blood sample, carried on foot with TB to the lab and immediately treated. TB: carried on foot with TA, stored not centrifuged, enveloped with Carbhypad® until TC arrival; control for the effects of waiting time before centrifugation on ammonia concentration results. TC: carried to the lab by PTS; control for the effects of pneumatic transportation*

Table 2: Effects on ammonia concentration results, of the pneumatic tube system (PTS) and of the elapsed time before blood sample centrifugation and assay [(TA; TB; TC) n=20]. *TA: referent blood sample, carried on foot with TB to the lab and immediately treated. TB: carried on foot with TA, stored not centrifuged, enveloped with Carbhypad® until TC arrival; control for the effects of waiting time before centrifugation on ammonia concentration results. TC: carried to the lab by PTS; control for the effects of pneumatic transportation.*

Results were compared using **Passing- Bablok** linear regression. **A**=accepted, **R**=refused, attributed to each of the three concentrations, 50, 100, 200 µmol/l [28]. Significance of paired t- test: $p < 0.050$.

Table 3: Protocol 2-1. Effects on ammonia concentration results, of different waiting times at +4°C of the centrifuged primary tubes and of the storage of separated plasma at +4°C (n=41). *PT0: first ammonia concentration measured on the primary tube; PT1 2-1: second ammonia concentration measured one hour later on the same primary tube, recapped and stored at +4°C; PT3: third ammonia concentration measured after three hours more of storage of primary tube, recapped at +4°C; AL3: ammonia assay on plasma separated after the PT1 2-1 measurement (n=41) and stored three hours at +4°C.*

Protocol 2-2: Ammonia concentration results after plasma storage for a week at -20°C and -80°C. *Plasma separated after PT1 2-2 measurement (n=47 minus two outliers).*

Results were compared using linear regression of **Passing- Bablok**. **A** means accepted, **R** means refused attributed to each of the three different levels of ammonia concentration 50, 100, 200 $\mu\text{mol/L}$ [28].

Significance of paired t- test: $p < 0.05$.

Figures

Figure 1: Flow charts of the sample analysis pathways in protocols 1 (A) and 2 (B).

Protocol 1 (A): Effects on ammonia concentration results, of the pneumatic system (PTS) and of the elapsed time between blood sample collection and assay [(TA, TB, TC) $n=20$]. **TA:** *referent blood sample*. **TB:** *control tube for the effects of waiting time before centrifugation on ammonia concentrations*. **TC:** *control tube for the effects of pneumatic transportation on ammonia concentrations*.

Protocol 2 (B)- protocol 2-1: Effects on ammonia concentration results, of different waiting times at $+4^\circ\text{C}$ of the centrifuged primary tubes and of the storage at $+4^\circ\text{C}$ of separated plasma.

PT0: *first ammonia concentration measured on the primary tube*; **PT1 (PT1 2-1 $n=41$):** *second measurement one hour at $+4^\circ\text{C}$ later on the same primary tube*. **PT3:** *third measurement after three hours more of storage at $+4^\circ\text{C}$* . **AL3:** *ammonia concentration on plasma separated after PT1 measurement and three hours of storage at $+4^\circ\text{C}$* .

- **protocol 2-2:** Ammonia concentration results after plasma storage for a week at -20°C and -80°C . Plasma was separated after PT1 measurement on $n=49$ specimens (PT1 2-2).

Figure 2: Protocol 1 boxplots: Effects on ammonia concentration results, of the pneumatic tube system (PTS) and of the elapsed time before blood sample centrifugation and assay [(A, B, C) $n=20$]. **TA:** *referent blood sample*. **TB:** *control tube for the effects of waiting time before centrifugation on ammonia concentrations*. **TC:** *control tube for the effects of pneumatic transportation on ammonia concentrations*.

Average is represented by the plus symbol; significance of paired t- test: $*p < 0.050$, $**p < 0.010$, $***p < 0.001$

Figure 3- A: Protocol 2-1 boxplots: Effects, on ammonia concentration results, of different waiting times at $+4^\circ\text{C}$ of the primary centrifuged tubes and of the storage at $+4^\circ\text{C}$ of the separated plasma ($n=41$). **PT0:** *first ammonia concentration measured on the primary tube*; **PT1 2-1:**

second measurement one hour at +4°C later on the same primary tube. **PT3**: third measurement after three hours more of storage at +4°C. **AL3**: ammonia concentration on plasma separated after PT1 2-1 measurement and three hours of storage at +4°C.

Blood samples (TA, TB, TC n=20)	Median time periods (min.-max.) in minutes since blood collection			
	<i>Until tube arrival at the lab</i>	<i>Until registration in the LIS (patient identity, analysis request)</i>	<i>Until tube centrifugation</i>	<i>Until ammonia assay</i>
Tubes A+B	4.5 (2.0-8.0)			
Tube A		4.5 (2.0-8.0)	6.0 (4.0-10.0)	19.0 (14.0-35.0)
Tube C	35.0 (19.0- 108.0)			
Tubes B+C		43.0 (25.0- 159.0)	58.0 (34.0- 170.0)	74.0 (45.0- 208.0)

- **B: Protocol 2-2 boxplots: Ammonia concentration results after plasma storage at -20°C and -80°C (n=47).** PT1 2-2 referent ammonia concentration.

Average is represented by the plus symbol; significance of paired t-test: *p<0.050, **p<0.010, ***p<0.001

Table 1: Median time periods, expressed in minutes, between the blood collection and the different steps of sample treatment in the lab until the ammonia assay [(TA, TB, TC) n=20].

Tube A (TA): referent blood sample, carried on foot with TB to the lab and immediately treated. Tube B (TB): carried on foot with TA, stored not centrifuged, enveloped with Carbhypad® until TC arrival; control for the effects of waiting time before centrifugation on ammonia concentration results. Tube C (TC): carried to the lab by PTS; control for the effects of pneumatic transportation

Blood samples- tubes	TA	TB	TC
Mean \pm SD ($\mu\text{mol/l}$)	50.9 \pm 27.4	53.1 \pm 29.9	56.5 \pm 29.5
SE	6.1	6.7	6.6
95%CI)	32.0 - 65.0	39.2 - 67.1	42.7 - 70.3
Median ($\mu\text{mol/l}$) (min.-max.)	45.5 (13.0-110.0)	46.0 (16.0-121.0)	52.0 (20.0-123.0)
Difference in results	TB vs. TA	TC vs. TA	TC vs. TB
Mean bias (%)	+5.1	+15.2	+10.6
Mean difference \pm SD ($\mu\text{mol/l}$)	2.3 \pm 6.4	5.6 \pm 7.3	3.3 \pm 6.6
SE of difference	1.4	1.6	1,5
95% confident interval (95%CI)	- 0,7 to 5.2	2.1 to 9.0	0.2 to 6.4
Paired t-test p-value	0.130	0.003	0.039
Comparative study Passing- Bablok linear regression	TB vs. TA	TC vs. TA	TC vs. TB
<ul style="list-style-type: none"> • Slope [95% CI] • Intercept [95% CI] • Correlation coefficient • SFBC conclusions 	1.04 [0.98 to 1.24] - 0.9 [-8.7 to 2.4] 0.98 AAA	1.06 [0.89 to 1.26] 3.4 [-5.8 to 11.4] 0.97 AAA	1.00 [0.88 to 1.10] 3.5 [-0.9 to 11.2] 0.98 AAA

Table 2: Effects on ammonia concentration results, of the pneumatic tube system (PTS)

and of the elapsed time before blood sample centrifugation and assay [(TA; TB; TC) n=20]. **TA: referent blood sample**, carried on foot with TB to the lab and immediately treated. **TB: carried on foot with TA**, stored, not centrifuged, enveloped with Carbhypad® until TC arrival; control for the **effects of waiting time before centrifugation** on ammonia concentration results. **TC: carried to the lab by PTS**; control for the **effects of pneumatic transportation**.

Results were compared using **Passing- Bablok linear regression**. **A: Accepted, R: Refused**, attributed to each of the three different levels of ammonia concentrations, 50, 100, 200 $\mu\text{mol/l}$ [28]. Significance of paired t- test: $p < 0.050$.

Blood samples	Protocol 2-1: waiting times (n=41)				Protocol 2-2: plasma freezing storage (n=49)				
	PT0	PT1 2-1	PT3	AL3	PT1 2-2	plasma at -20°C	plasma at -80°C		
Mean ±SD (µmol/l)	51.6 ± 41.9	56.1 ± 42.3	77.8 ± 45.0	65.4 ± 42.5	60.0 ± 45.9	72.9 ± 61.3	66.2 ± 57.9		
SE	6.5	6.6	7.0	6.6	6.6	8.8	8.3		
95% Confidence Interval	38.4- 64.8	43.0 - 69.6	63.6 - 92.0	51.9 - 78.8	46.8 - 73.1	55.3 - 90.5	49.5 - 82.3		
Median (µmol/l) (min.-max.)	40.0 (10.0 - 217.0)	42.0 (10.0- 221.0)	62.0 (26.0- 274.0)	52.0 (17.0- 219.0)	47.0 (10.0-228.0)	55.0 (18.0-381.0)	49.0 (12.0- 338.0)		
Difference in results		PT12-1 vs. PT0	PT3 vs. PT0	AL3 vs. PT0		-20°C vs. PT12-2	-80°C vs. PT12-2		
Mean bias vs. PT0 (%)		+ 12.5	+ 80.0	+39.1		n=49 +23.9	n=47 +21.5	n=49 +8.8	n=47 +6.2
Mean difference ± SD (µmol/L)		4.4 ± 5.4	26.2 ± 11.6	13.7 ± 8.6		12.9 ± 24.8	8.3 ± 8.3	6.2 ± 20.1	2.3 ± 6.5
SE of difference		0.8	1.8	1.3		3.5	1.2	2.9	0.9
95% Confidence Interval		2.7 - 6.1	22.5 - 29.8	11.0 - 16.5		5.8- 20.0	5.9- 10.7	0.4- 12	0.4- 4.2
paired t-test p-value		<0.0001	<0.0001	<0.0001		0.0007	<0.0001	0.036	0.017
Comparative study Passing- Bablok		PT12-1 vs. PT0	PT3 vs. PT0	AL3 vs. PT0		-20°C vs. PT12-2	-80°C vs. PT12-2		
<ul style="list-style-type: none"> Slope [95%CI] Intercept [95%CI] Correlation <i>coeff.</i> SFBC conclusions 		1.03 [2.0 to 1.1] 2.7 [- 1.1 to 4.9] 0.99 AAA	1.08 [1.0 to 1.2] 19.9 [14.1 to 26.2] 0.97 RRR	1.10 [1.0 to 1.2] 9.3 [3.5 to 13.0] 0.98 RRR		n=49 1.05 [0.99 to 1.18] 6.21 [0.82 to 8.39] 0.93 RAA	n=49 1.04 [0.98 to 1.15] 1.44 [-3.3 to 5.3] 0.95 AAA		
<ul style="list-style-type: none"> Slope [95%CI] Intercept [95%CI] Correlation <i>coeff.</i> SFBC conclusions 						n=47 1.02 [0.96 to 1.12] 6.60 [3.48 to 10.0] 0.99 RAA	n=47 1.01 [0.96 to 1.09] 2.56 [-1.18 to 5.06] 0.99 AAA		

Table 3: - Protocol 2-1. Effects on ammonia concentration results, of different waiting times at +4°C of the centrifuged primary tubes and of the storage at +4°C of separated plasma (n=41). PT0: first ammonia concentration measured on the primary tube; PT1 2-1: second ammonia concentration measured one hour later on the same primary tube, recapped and stored at +4°C; PT3: third ammonia concentration measured after three hours more of storage of primary tube, recapped, at +4°C; AL3: ammonia concentration of plasma, separated after PT12-1 measurement (n=41) and stored three hours at +4°C.

- Protocol 2-2: Ammonia concentration results after plasma storage for a week at -20°C and -80°C. Plasma separated after PT1 2-2 (n=47 minus the two outliers).

Results were compared using **Passing- Bablok linear regression**. **A** = accepted, **R** = refused, attributed to each of the three different levels of ammonia concentrations, 50, 100, 200 µmol/l [28]. Significance of paired t- test: p<0.050.

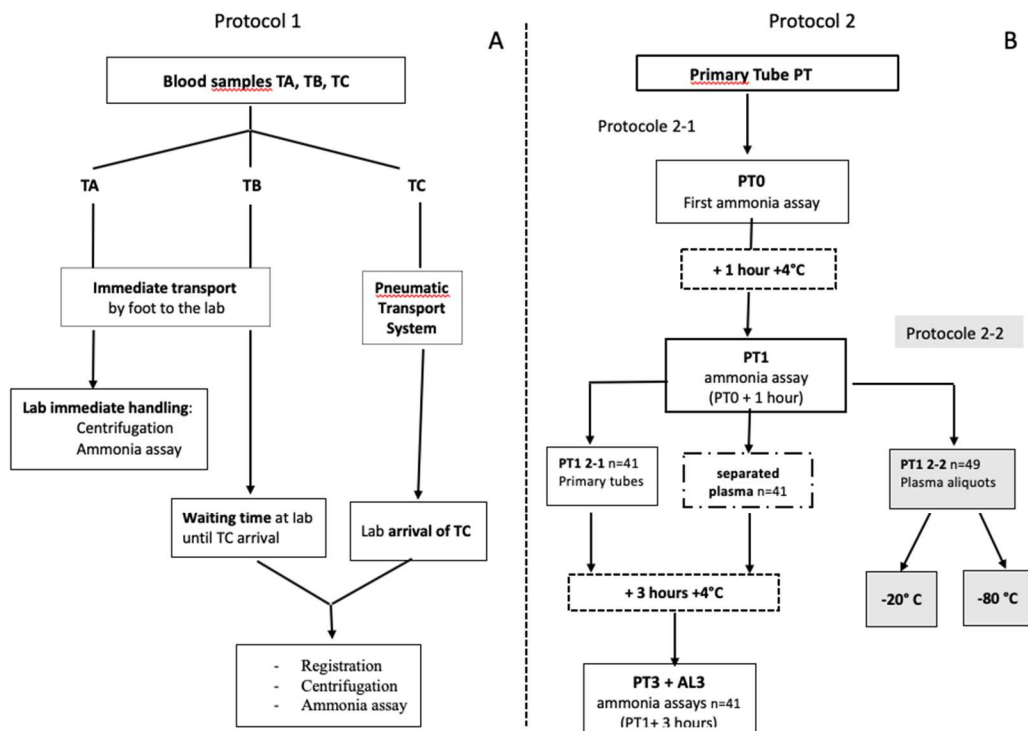


Figure 1: Flow charts of the sample analysis pathways in protocols 1 (A) and 2 (B).

Protocol 1 (A): Effects on ammonia concentration results, of the pneumatic system (PTS) and of the elapsed time between blood sample collection and assay [(TA, TB, TC) n=20]. **TA:** referent blood sample. **TB:** control tube for the effects of waiting time before centrifugation on ammonia concentrations. **TC:** control tube for the effects of pneumatic transportation on ammonia concentrations.

Protocol 2 (B)- protocol 2-1: Effects on ammonia concentration results, of different waiting times at +4°C of the centrifuged primary tubes and of the storage at +4°C of separated plasma.

PTO: first ammonia concentration measured on the primary tube; **PT1 (PT1 2-1 n=41):** second measurement one hour at +4°C later on the same primary tube. **PT3:** third measurement after three hours more of storage at +4°C. **AL3:** ammonia concentration on plasma separated after PT1 measurement and three hours of storage at +4°C.

- protocol 2-2: Ammonia concentration results after plasma storage for a week at -20°C and -80°C. Plasma was separated after PT1 measurement on n=49 specimens (PT1 2-2).

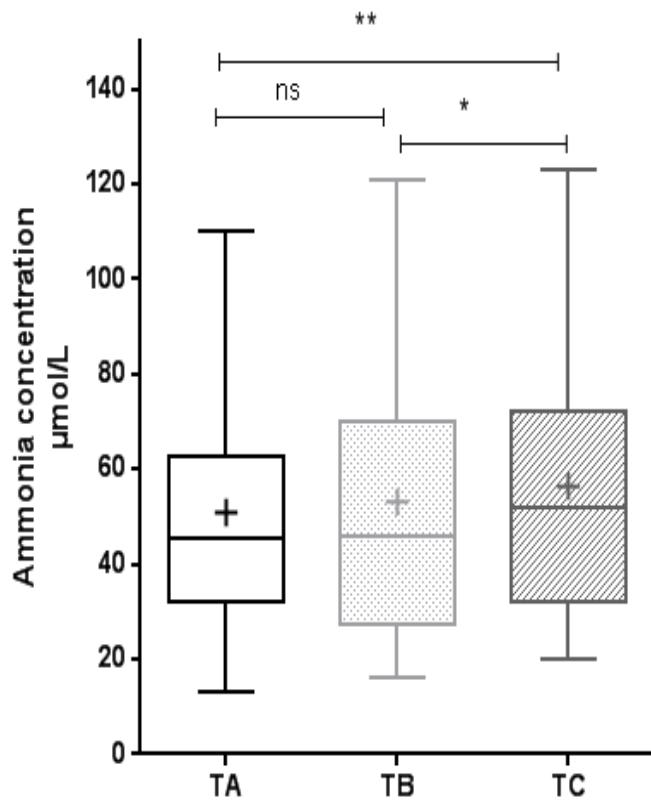


Figure 2: Protocol 1 boxplots: Effects on ammonia concentration results, of pneumatic tube system (PTS) and of elapsed time before blood sample centrifugation and assay [(TA; TB; TC) n=20]. *TA: referent blood sample. TB: control tube for the effects of waiting time before centrifugation on ammonia concentration results. TC: control tube for the effects of pneumatic transportation on ammonia concentrations.*

Average is represented by the plus symbol; significance of paired t-test: *p<0.050, **p<0.010, ***p<0.001

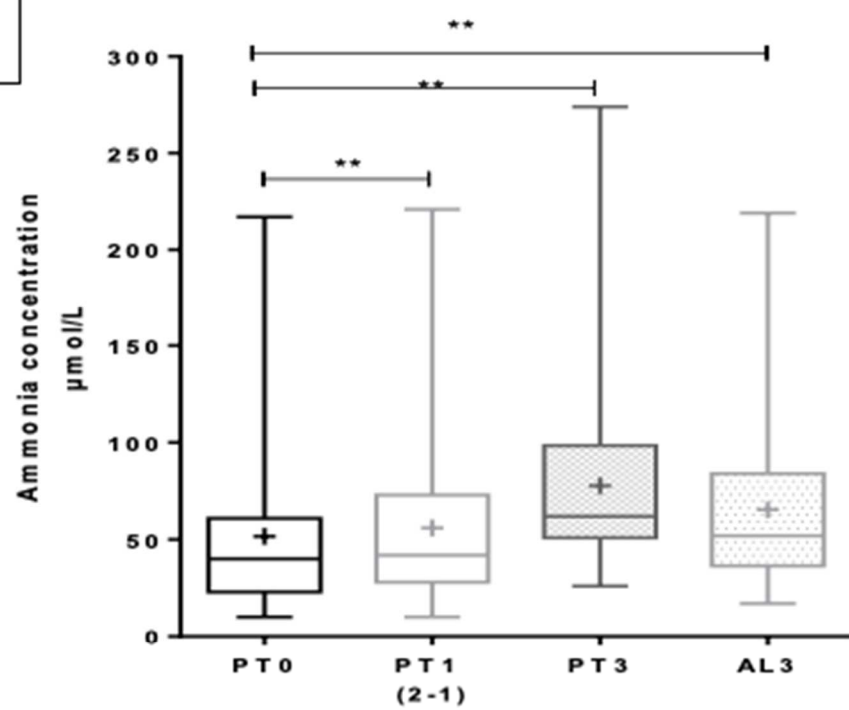
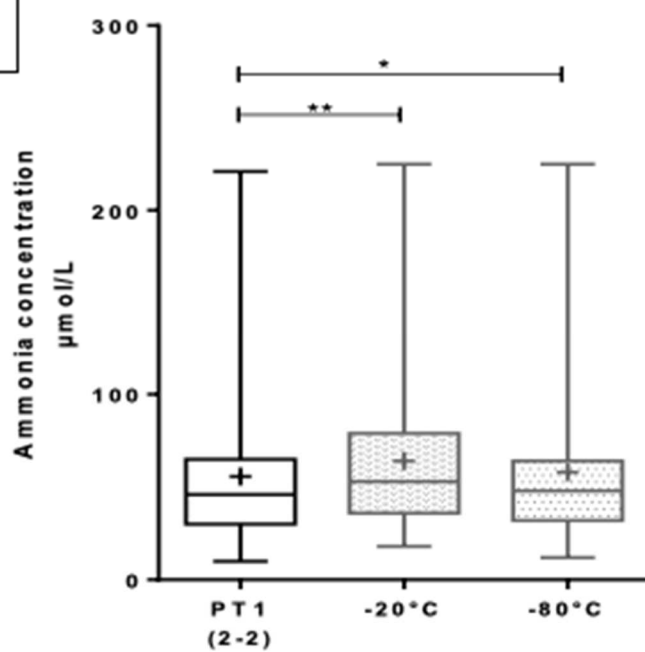
A**B**

Figure 3: A- Protocol 2-1 boxplots. Effects on ammonia concentration results, of different waiting times at +4°C of the centrifuged primary tubes and of storage at +4°C of the separated plasma (n=41).

PT0: first and referent ammonia concentrations measured on the primary tube; PT1 2-1: second measurement one hour at +4°C later on the same primary tube. PT3: third measurement after three hours more of storage at +4°C. AL3: ammonia concentrations on plasma separated after PT12-1 measurements and three hours of storage at +4°C.

: B- Protocol 2-2 boxplots. Ammonia concentration results after plasma storage for one week at -20°C and -80°C (n=47). PT1 2-2 referent ammonia concentrations.

Average is represented by the plus symbol; significance of paired t -test: *p<0.050, **p<0.010, ***p<0.001