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Avoiding falsely low creatinine concentrations measured in patients treated with N-acetylcysteine for acetaminophen intoxication using enzymo-amperometric method – An *in vitro* and *in vivo* study

Running title: Enzymo-amperometric methods prevent N-acetylcysteine interference on creatinine assays

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Abstract

Background: Circulating creatinine is a biomarker of paramount importance in clinical practice. In cases of acetaminophen (APAP) intoxication, the antidote, N-acetylcysteine (NAC), interferes with commonly used creatininase-peroxidase methods. This study aimed to assess whether creatininase-amperometric methods were affected in this context.

Methods: This study includes *in vitro* interference tests, involving four creatinine assays using NAC-spiked plasma pools and an *in vivo* retrospective study comparing creatininase-peroxidase and creatininase-amperometric measurements in patients presenting with NAC-treated APAP poisoning.

Results: Creatininase-peroxidase method was impacted by NAC interference in a clinically-significant manner at therapeutic NAC levels (basal value recovery of 80% and 70% for 500 and 1000 mg.L⁻¹ of NAC, respectively), surpassing the desirable Reference Change Value (RCV%). Enzymo-amperometric methods were not impacted. Among patients, a mean bias of -45.2 ± 28.0 % was observed for the peroxidase detection method compared to the amperometric in those who received NAC prior plasma sampling and -2.7 ± 5.4 % in those who did not.

Conclusions: Our findings indicate that enzymo-amperometric creatinine assays remain unaffected by NAC interference due to the absence of the peroxidase step in the analytical process. Therefore, these methods are suitable to prevent spurious hypocreatinemia in APAP intoxicated patients undergoing NAC therapy.

Keywords: Acetaminophen intoxication, N-acetylcysteine, peroxidase, analytical interference, creatinine assay

1. Introduction

Circulating creatinine is a by-product of the non-enzymatic metabolism of muscular creatine [1]. The total body content of creatine being constant, there is a continuous production of creatinine, which is excreted in urine. Despite several limitations, such as a delayed elevation allowing to detect renal damage, it remains the gold-standard biomarker for assessing renal function, as it is a specific glomerulus-filtered molecule that is affordable to test [2]. As a result, creatinine measurement is one of the most ordered laboratory tests [3,4]. Regarding biological variation, the within-subject biological variation of creatininemia is rather low, depending on meat consumption, and is higher in people without chronic kidney disease (CKD) compared to people with CKD (6.4% vs 2.5%). Therefore, in the absence of sudden acute kidney injury, creatininemia shall remain stable within a patient, regardless of the renal status [5]. Nowadays, creatininemia is usually measured using either Jaffe's alkaline picrate method or enzymatic-colorimetric assays relying on creatininase and horseradish peroxidase (Trinder reaction). The latter have proven to be less prone to interference and therefore tend to be democratized [6].

Acetaminophen (APAP or paracetamol) ranks among the most widely consumed analgesic drugs in Europe, with ever increasing sales [7,8]. It serves as a secure primary choice for analgesic and antipyretic treatment when following the recommended doses, which has led to its widespread use. Nevertheless, even at therapeutic concentration, acetaminophen undergoes partial (5-10%) oxidation into a highly reactive electrophile metabolite, N-acetyl-p-benzoquinone-imine (NAPQI), through the action of cytochrome P450 2E1 (4-nitrophenol 2-hydroxylase, EC 1.14.13.n7) [9]. NAPQI is subsequently detoxified through conjugation to glutathione, resulting in its excretion as cysteine derivatives [10]. In case APAP overdose, this detoxification pathway becomes overwhelmed, giving rise to APAP poisoning, which is common and potentially lethal [11]. This occurrence primarily rises from APAP's accessibility as an over-the-counter medication, widely available without prescription in numerous countries, and exacerbated by the fact that the toxic dose is just twice the maximum daily recommended therapeutic dose. As a result, acetaminophen is involved in hundreds of deaths and much more hospitalizations annually in the event of iatrogenic overdoses or suicide attempts in western countries [12]. To date, APAP poisoning management continues to involve the administration of an antidote, intravenous N-acetylcysteine (NAC), when circulating APAP levels exceed $150 \text{ mg}\cdot\text{L}^{-1}$, following the recommendations established by Prescott in 1979 [13].

NAC functions as an antioxidant medication, known to be a substrate of horseradish peroxidase, a reagent employed in Trinder-based analytical methods such as enzymatic-colorimetric assays for creatinine, total cholesterol or uric acid [14–16]. Specifically, a negative interference on creatininase-peroxydase based assays by NAC

has been scarcely reported *in vitro* and in patients [17–20]. As illustrated in **Figure 1**, it is postulated that NAC leads to an underestimation of creatinine by inhibiting H₂O₂ oxidation by peroxidase, consequently yielding a diminished quinone-imine chromogen output [17]. Thus, the reported creatinine result, directly proportional to the light absorption, is spuriously low. It is consistent with the Jaffe's alkaline picrate colorimetric method not being impacted, given that this method does not involve peroxidase [18]. Over recent years, whole-blood creatinine assays have been developed to provide a short turnaround time rendering of circulating creatinine in an emergency context, for example to evaluate renal status before an imaging examination with contrast medium [21,22]. These assays, suitable for laboratories with limited activity or for a point-of-care setting, rely on enzymo-amperometric methods using the same enzymatic sequence but featuring H₂O₂ reduction by a platinum electrode for signal detection instead of a dye formation by horseradish peroxidase [23]. To our knowledge, these enzymo-amperometric methods have not been evaluated in a context of APAP poisoning. The present study aimed to investigate the potential impact of therapeutic concentrations of N-acetylcysteine on four creatinine methods to assess whether this interference could jeopardize the renal function estimation in APAP intoxicated patients and if enzymo-amperometric methods could represent a solution. In addition, we strengthen these *in vitro* findings with retrospective data from our centre.

2. Material and methods

2.1. In vitro study

2.1.1. Study design

Different creatinine levels (50, 100 and 200 $\mu\text{mol.L}^{-1}$) pools were made with left-over plasma from routine diagnosis leftovers from inpatients and outpatients of Rennes University Hospital Centre. Briefly, blood samples for chemistry analysis were collected by peripheral venepuncture on BD vacutainer[®] lithium heparin blood collection tubes with separating gel (Beckton-Dickinson, Franklin Lakes, NJ, USA). Plasma was then obtained through samples centrifugation (10 min, 1885 g at room temperature). Samples selection aimed to minimize interferences, consequently, only samples that exhibited no hemolysis, icterus, or lipemia, as determined by the HIL test on the Roche cobas[®] analyzer, were included in the study. These pools were assayed for the trinder-based creatinine assay. They were then spiked with increasing levels of N-acetylcysteine (Hidonac[®], Zambon France S.A., Issy-les-Moulineaux, France) in order to mimic different therapeutic concentrations, i.e. 0 (NAC_0), 50 (NAC_{50}), 500 (NAC_{500}) and 1000 (NAC_{1000}) mg.L^{-1} , according to the pharmacokinetics data outlined in the summary of product characteristics [24]. The prepared samples were then assayed for creatininase-peroxidase creatinine, Jaffe's creatinine, and the two enzymo-amprometric creatinine methods.

For NAC_{1000} concentration, an additional set of nine supplemental independent samples with creatinine concentration ranging between 100 and 200 $\mu\text{mol.L}^{-1}$ were spiked with NAC, tested across all methods and compared to their NAC_0 results. For each level of NAC concentrations, bias (%) between the $\text{NAC}_{50/500/1000}$ and the NAC_0 levels was expressed as follows:

$$\text{Bias}\% = 100 * \frac{([\text{analyte}]_{\text{NAC } 50/500/1000} - [\text{analyte}]_{\text{NAC } 0})}{[\text{analyte}]_{\text{NAC } 0}}$$

In the results, data are either expressed as bias (%) from the baseline value or as the percentage (%) of recovery relative to the baseline value established for the NAC_0 level (i.e. $100 - \text{Bias}\%$).

2.1.2. Creatinine measurement

As illustrated in **Figure 1**, creatinine was measured using four different IDMS-traceable methods performed on three analyzers: i) An enzymatic-based method using a creatininase-creatinase-sarcosine oxydase-peroxydase sequence with a spectrophotometric detection of a quinone imine chromogen at 700/546 nm on Roche

cobas® 8000 (Hoffmann-La Roche, Basel, Switzerland); ii) A picric acid-based colorimetric method (Jaffe's) with a spectrophotometric detection at 512/583 nm of a yellow-orange complex (Janowski complex) formation at alkaline pH, on cobas® as well; and iii) An enzymo-amperometric method using a creatininase (-creatinase-sarcosine oxydase) but with a amperometric detection of H₂O₂ by a platine anode instead of a peroxidase-formed chromogen. This method was performed both on Radiometer ABL90 FLEX PLUS® (Radiometer, Copenhagen, Denmark) and Abbott i-STAT® 1 analyzers (Abbott, Chicago, IL, USA).

2.1.3. Assessment of NAC interference impact from a clinical standpoint

As recommended by the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), the clinical impact of the NAC interference on creatinine was evaluated according to the Reference Change Value (RCV%) formula: $RCV\% = Z \cdot \sqrt{2} \cdot \sqrt{(CV_A^2 + CV_I^2)}$ [25], where Z is 1.96 for a one-sided p value of 0.025, CV_A is the analytical variation, and CV_I is the within-subject variation [26]. Based on our laboratory analytical performances, for creatinine, desirable median RCVs value was 12.2% (95% CI: 11.6-15.1%) for a decrease and 13.9% (95% CI: 13.2-17.7%) for an increase. Therefore, for each level of NAC, a basal value recovery for creatinine inferior to: 100 – median RCV% decrease (i.e. inferior to (100 – 12.2 = 87.8%)) or superior to 100 + 13.9 = 113.9% was considered significant from a clinical standpoint.

2.2. Retrospective data collection

We gathered retrospective data from our Laboratory Information System (LIS) of patients admitted to Rennes University Hospital Centre from May 2021 to January 2023 in a context of acetaminophen intoxication, selecting those with a reported APAP level over 150 mg.L⁻¹, corresponding to the systematic antidotic treatment threshold. In these patients, plasma were obtained following the aforementioned sampling procedure and whole blood specimens for point of care (POC) creatinine measurement were obtained by radial artery puncture on Radiometer safePICO heparinized syringes (Radiometer, Copenhagen, Denmark).

Twelve patients were included in the study. APAP levels measured on Roche cobas® 8000 (G6PDH-based immunoenzymatic method) were reported for all patients. Epidemiological and clinical information was anonymized. The following timeframes were reported: the time elapsed from acetaminophen ingestion to the initiation of NAC treatment and the time elapsed from the initiation of NAC to measurement of circulating creatinine. As a result, two groups were identified. The first group, referred to as the 'NAC-treated' group (n= 8) consisted of patients who received NAC before the blood sample was drawn. The second group did not received NAC yet at the time of blood sampling (n= 4). In both groups, plasma creatinine levels were measured on Roche

cobas® 8000 analyzer and simultaneous whole blood creatinine levels measured on Radiometer ABL90 FLEX PLUS®. Results were compared to assess the extent of the interference caused by NAC treatment.

2.3. Statistical analyses

Statistical analyses and figures were performed with Graphpad Prism 10.0.2 software (San Diego, CA, USA). Variables distributions were tested for normality through a Shapiro-Wilk test. As distributions were not normal, difference between paired data was tested through Wilcoxon matched-pairs signed rank test and correlation was tested with Spearman's correlation test. P values <0.05 were considered as statistically significant.

3. Results

3.1. In vitro study

3.1.1. NAC interference on creatinine

The impact of NAC on cobas® enzymatic-colorimetric creatinine assay was found to be dependant of the NAC concentration, regardless of the creatinine level. The mean bias was -4.8 % (min: -2.9%; max: -5.9%) for the NAC₅₀ mg.L⁻¹ level, -20.1% (-19.0%; -21.2%) for the NAC₅₀₀ mg.L⁻¹ level and -30.5% (-28.8%; -31.4%) for the NAC₁₀₀₀ mg.L⁻¹ level. Bias correlation to NAC concentration was found significant (**Figure 2A**, Spearman's $\rho = -0.89$ ($|\rho| > 0.7$ defines a very strong correlation), P value <0.0001). The plot comparing the NAC₀ and NAC₁₀₀₀ levels on the three pools and the nine supplemental samples showed a mean bias of -28.0% (95% CI: -38.5%; -26.5%), which was constant along the creatinine concentration and confirmed that the bias was independent of the creatinine concentration (**Figure 2B**).

As shown in **Figure 3**, we evaluated the four creatinine assays using the creatinine pools spiked with increasing levels of NAC. **Figure 3A** displays the difference of creatinine measure between the enzymo-colorimetric assay on Roche cobas® (set as the control) and the three other methods without NAC. On **Figure 3B**, the impact of increasing levels of NAC on the four methods is illustrated, each one being compared to its own baseline level (NAC_0). Jaffe's method and the i-STAT® 1 method were not affected by NAC, even at the NAC_{1000} level. However, they both showed an overestimation of the baseline creatinine value (mean: 128%, ranging from 113% to 144% for Jaffe's and mean: 113%, ranging from 98% to 118% for i-STAT® 1) compared to the creatininase assay, Jaffe's method exceeding the positive RCV (113.9%). The ABL90 method displayed the most reliable baseline value recovery compared to creatininase for the NAC_0 level and was not affected by NAC as well. To be noted that the cobas® creatininase method exceeded the desirable RCV for a decrease (87.8%) at the NAC_{500} (80%) to NAC_{1000} (70%) levels.

3.2. Retrospective data

In patients, the average APAP level was 247 mg.L⁻¹ (min: 157 mg.L⁻¹; max: 427 mg.L⁻¹). The mean time from acetaminophen ingestion to the initiation of NAC treatment was 5.1 ± 3.0 hours in 'NAC-treated' group and 7.9 ± 3.5 hours in 'no NAC-treated' group. Within the 'NAC-treated group', the mean time from N-acetylcysteine treatment initiation to the circulating creatinine measurement was 2.5 hours (min: 0.25 h; max: 7.0 h). The Wilcoxon matched-pairs signed rank test showed that creatinine levels measured with enzymatic-colorimetric method on cobas® 8000 were significantly lower (median decrease: -36.9%, range: -14.9% to -93.8%) than the corresponding levels measured with enzyme-amperometric method on ABL90 in the 'NAC-treated group' (P= 0.0078, **Figure 4A**), while levels were not different (median variation: -3.4%, range: -8.1% to +4.3%) in the 'no NAC-treated group' (P= 0.3750, **Figure 4B**).

4. Discussion

This study presents evidence regarding the potential clinical impact of the N-acetylcysteine on circulating creatinine measurement. APAP intoxication is suspected in patients reporting the ingestion of 4 g of APAP or more within 8 hours [27] or presenting a plasma APAP level exceeding $150 \mu\text{g.L}^{-1}$ at 4 hours after ingestion [28]. The categorization of massive acetaminophen ingestion (newly labelled 'high-risk') varies among authors and is based either on the ingested dose (>30, 40 or 50g) or on the plasma APAP level surpassing a specific threshold, such as 250 or $500 \mu\text{g.L}^{-1}$ [29]. The management of APAP intoxication is typically structured and follows a three-step, 21-hour sequence of intravenous NAC administration: initially, a loading dose of 150mg.kg^{-1} over 1h, followed by a second maintenance dose of 50mg.kg^{-1} over 4h and finally a third dose of 100mg.kg^{-1} over 16h [30]. Using this protocol, mean plasma NAC concentrations of 554mg.L^{-1} (range $304 - 875 \text{mg.L}^{-1}$) are achieved [31]. These concentrations, as indicated by our findings (**Figure 2B**, mean bias = -32.5% at $\text{NAC}=1000 \text{mg.L}^{-1}$) and those reported by others [17,18] are sufficient to jeopardize Trinder-based creatinine assays in a concentration-dependent manner (**Figure 2A**, $\rho = -0.89$, $P < 0.0001$), as NAC is a competitive inhibitor of horseradish peroxidase (**Figure 1**). On the other hand, oral NAC treatment, sometimes used in APAP intoxication and advocated in other indications such as chronic kidney disease or chronic obstructive pulmonary disease [32,33] is not likely to impact creatinine assays given the low plasmatic levels reach by this route of administration ($\sim 3 \text{mg.L}^{-1}$) [34]. Furthermore, recent publications have reviewed current data on APAP poisoning, suggesting a reevaluation of NAC protocols, with a more patient-tailored perspective. For instance, Hendrickson *et al.* emphasized that, given that the final infusion rate of $6.25 \text{mg.kg}^{-1}.\text{h}^{-1}$ of NAC was determined for an ingestion of 16 g of APAP. Thus, a proportional increase of double, triple or even quadrupled doses would be warranted in the event of higher ingested doses (i.e. 32, 48 or 64 g) [35]. A consensus statement for the management of APAP poisoning in the US and Canada also recommends discussing the NAC dose escalation with the poison centre or a clinical toxicologist in case of high-risk APAP ingestion. However, authors underlie the insufficiency of medical literature on this matter [36]. Consequently, the circulating NAC levels should raise along with the posology. Thus, the interference of NAC on all Trinder-based assays, including creatinine, is likely to become more common in the future. It should be noted that other analytes, such as triglycerides, uric acid, cholesterol or total and HDL-cholesterol, which rely on Trinder-based assays, are not typically part of the initial evaluation for APAP poisoning and, therefore, are less likely to mislead clinicians in patient management. That being said, it is important to highlight that the aforementioned NAC protocol is also administrated in the context of early stage non-acetaminophen acute liver failure, as it has been associated with improved transplant-free survival [37]. In these patients, NAC interference can indeed compromise laboratory results for the mentioned analytes and potentially result in mismanagement. In addition, it is worth

noting that creatinine is part of the Model for End-Stage Liver Disease (MELD) score, a scale used to assess the mortality risk in individuals with end-stage liver disease and supporting organ allocation priorities [38]. In this context, inaccuracies in creatinine measurements may have severe consequences, such as misclassifying a patient on the transplant waiting list.

From an analytical perspective, it's important to note that despite considerable efforts to standardize creatinine assays by calibrating them to isotope dilution mass spectrometry (IDMS) and using the standard reference material (SRM) 967, not all creatinine methods are strictly equivalent. Jaffe's method, in particular, is known to be more susceptible to interferences and analytical variability. As a result, enzymo-colorimetric creatinine assays are progressively replacing Jaffe's method in medical laboratories [6,39]. Our results show a positive bias between alkaline picrate colorimetric and creatininase-peroxydase methods (**Figure 3A**, mean 128%, ranging from 113% to 144%, exceeding the desirable positive RCV%), which is consistent with literature and confirm the important inter-assay variability for creatinine measurement [40,41]. However, despite creatininase-based methods being less susceptible to other interferences, such as glucose, bilirubin or ketone bodies, compared to Jaffe's method [42], the latter performs better and is not affected by NAC treatment (**Figure 3B**).

In the *in vitro* part of our study, the main finding is that enzymo-amperometric methods are reliable for obtaining interference-free creatinine measurements with NAC, as they do not involve peroxidase. However, it is worth noting that the i-STAT® 1 analyzer tends to overestimate reference creatinine level (**Figure 3A**, mean 113%, ranging from 98% to 118%), a characteristic that has been previously documented [43]. Importantly, in this *in vitro* study, all methods were performed on plasma while the retrospective study involved paired-comparison between a plasma enzymo-colorimetric method and a whole-blood enzymo-amperometric method. However, any bias arising from matrix effect can be ruled out, as heparin whole blood and plasma/serum have been demonstrated to be equivalent for measuring circulating creatinine [43,44]. An important point to discuss is whether APAP itself or its toxic metabolite N-acetyl-p-benzoquinone-imine (NAPQI) can interfere with creatinine assays, in addition to NAC. In their respective field safety note, manufacturers such as Roche and Siemens (regarding interference on creatininase-peroxydase on cobas® and Atellica CH® analyzers, respectively) indicate that not only NAC, but also NAPQI can interfere with Trinder-based assays. However, our retrospective data contradict this point, as in our patients, only the 'NAC-treated' group displayed differences in paired creatinine measurements, despite presenting toxic levels of APAP. Moreover, NAPQI is highly reactive and thus, has an extremely short half-life as it rapidly conjugated with glutathione or covalently binds to protein adducts [45]. It is therefore unlikely that NAPQI interferes on the creatinine assay. It is worthy to note that the median creatinine decrease (-36.9%) in the 'NAC-treated' group aligns with the *in vitro* findings of NAC

interference at theoretical therapeutic concentrations. However, two patients within this group exhibit a much greater creatinine decrease (-81% and -93.8%, respectively) compared to the median reduction. This discrepancy may be due to a blood sampling close to the time after administration where the concentration is the highest (T_{max} , achieved approximately 10 minutes after administration [46]) or to pre-analytical error such as sampling through an IV catheter.

This study has several limitations. Firstly, the *in vivo* study involved a small number of patients. This is primarily due to the aim of presenting reliable data, and it is inherent to the retrospective study design. In addition, this study did not assess interference with other manufacturers creatininase-peroxidase and enzymo-amperometric assays, or with other known interfering substances like metamizole, ascorbic acid or catecholamines [17,47,48]. Even though our study is sufficient to demonstrate that the peroxidase part of the creatinine measure is responsible for this interference, expanding the assessment to include these elements could have strengthened these findings. Manufacturers' technical sheets provide data on NAC interference on their enzymo-colorimetric assays, which can be compared to our findings. For example, Siemens states that the Atellica[®] Enzyme Creatinine_3 (ECre3) method, containing 36,000 U.L⁻¹ of horseradish peroxidase and using an 8.5 μ L sample volume, may experience falsely low creatinine measurement with NAC concentrations above 375 mg.L⁻¹. The decrease is reported as -38.7% for a creatinine level of 743 μ mol.L⁻¹ and a -51% decrease for a creatinine level of 87 μ mol.L⁻¹ at a NAC concentration of 1,500 mg.L⁻¹. Similarly, the Vista[®] assay, with 1,000 U.L⁻¹ of horseradish peroxidase and a 2.7 μ L sample volume mentions interference of <10% for a level of creatinine of 88.4 μ mol.L⁻¹ at a NAC concentration of 450 mg.L⁻¹. In contrast, the Beckman Coulter Synchron[®] assay, using a 4,800 U.L⁻¹ horseradish peroxidase and a 10 μ L sample volume, suggests a -6.3% decrease in creatinine at a NAC concentration of 500 mg.L⁻¹. This is discrepant with our findings of a mean bias of -20.1% at the NAC₅₀₀ level on the Roche assay, which displays a slightly decrease sample volume (1.7 μ L vs 10 μ L) and horseradish peroxidase concentration (1,000 U.L⁻¹ vs 4,800 U.L⁻¹) compared to Beckman Coulter. The above-mentioned technical data highlight the substantial heterogeneity in the design of assays, with the concentration of horseradish peroxidase and the sample volume potentially playing a role in their sensitivity to NAC interference. Moreover, there is a lack of standardization in assessing analytical interferences, with varying concentrations of NAC used, ranging from infra-therapeutic levels such as 333 mg.L⁻¹ for Roche to highly elevated levels such as 1,500 mg.L⁻¹, unlikely to be achieved. A harmonization of NAC interference testing among IVD manufacturers with an appropriate common NAC level would enhance screening for interference not only for creatinine assays, but for all Trinder-based assays, such as cholesterol or uric acid. Given these reasons and the necessity for a 'real-life' setting evaluation, a prospective multicentric-multianalyzers study conducted on a large

cohort with actual patients NAC concentrations would be highly beneficial in addressing these limitations.

5. Conclusion

This study provides further evidence on the peroxidase-based interference of N-acetylcysteine in patients presenting with APAP intoxication. We recommend the following actions: i) Laboratory medicine specialists should communicate with NAC users, such as emergency doctors or hepatologists to make them aware of this potential interference; ii) Laboratory Information Systems should include a comment on laboratory reports for creatinine in the context of APAP poisoning and iii) whenever possible, clinicians should use enzymo-amperometric methods such as those developed for point-of-care testing in this context to monitor circulating creatinine levels.

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Informed consent: Not applicable.

Ethical approval: Not applicable.

Figures

Figure 1. Main analytical sequences for creatinine determination in Laboratory Medicine. Creatinine can be measured using either **Jaffe's reaction** based on creatinine conjugation to picric acid in alkaline conditions, or through enzymatic methods. Enzymatic methods share a **common enzymatic pathway** using a creatininase to produce hydrogen peroxide, which then undergoes an **amperometric detection** using a platinum electrode, or a **colorimetric detection** using horseradish peroxidase. The latter pathway is common to all Trinder-based assay, such as total cholesterol, uric acid, lactic acid or creatinine. This figure was made using ChemDraw® 21.0.0.

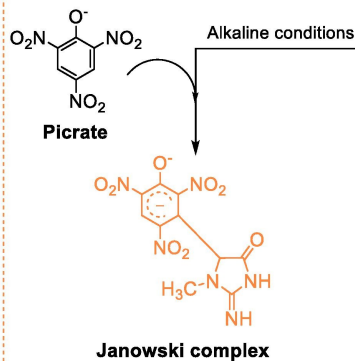
Figure 2. NAC interference on cobas® enzymatic-colorimetric creatininase method. **(2A)** Correlation between Bias% and NAC concentration. ρ : Spearman's correlation coefficient ($|\rho| > 0.7$ defines a very strong correlation). Desirable Reference Change Values (RCV%) threshold for significant clinical impact is noticed by the red dash line. **(2B)** Comparison plot featuring the % difference between creatinine levels measured with a NAC level of 0 and 1000 mg.L⁻¹ plotted against the NAC₀ creatinine value. Upper and lower 95% confidence interval of mean bias are plotted as dashed lines. On both graphs, plasma pools values are plotted as black dots and additional samples are plotted as black circled white dots.

Figure 3. Creatinine inter-assay variability and impact of NAC spiking on different creatinine assays. **(3A)** Difference between the creatininase-colorimetric assay and the three others. **(3B)** Each column displays the mean basal creatinine value recovery (%) for each level of NAC on each method compared to NAC₀. Desirable Reference Change Values (RCV%) thresholds for significant clinical impact are noticed by the red dash lines.

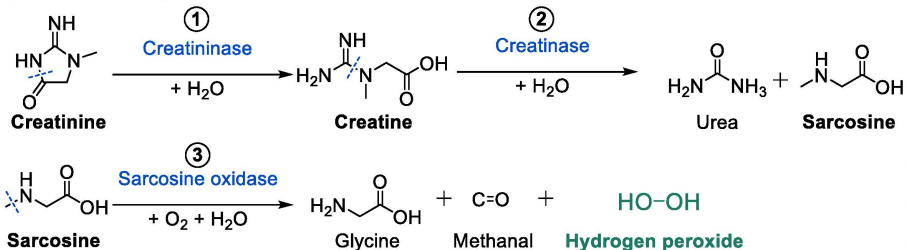
Figure 4. Wilcoxon matched-pairs signed rank test comparing creatinine levels measured by enzymatic-colorimetric method on lithium heparinate plasma and enzymo-amperometric method on lithium heparinate whole-blood, in patients who received NAC prior to blood sampling **(4A)** or did not **(4B)**. $P < 0.05$ is considered statistically significant.

Jaffe's reaction

Wavelength (sub/main) : 512/583 nm



Enzymatic pathway



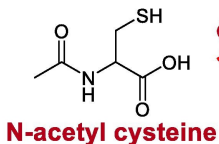
HO-OH
Hydrogen peroxide

Amperometric detection



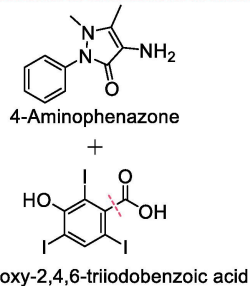
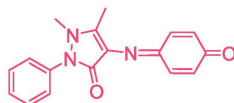
Colorimetric detection

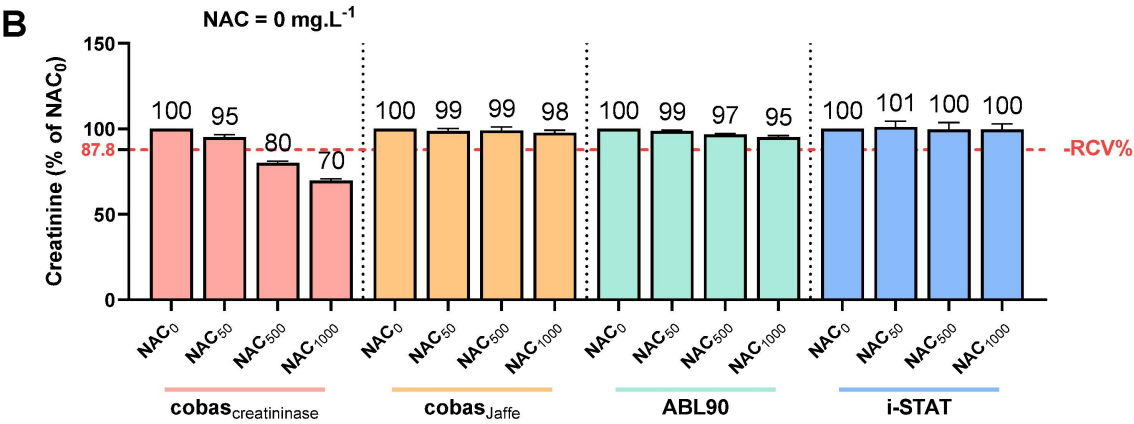
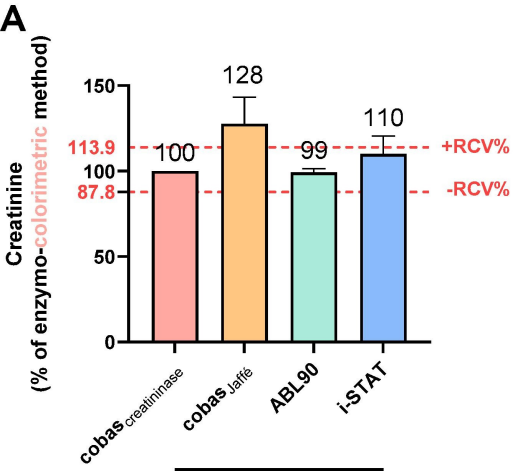
Wavelength (sub/main) : 700/546 nm

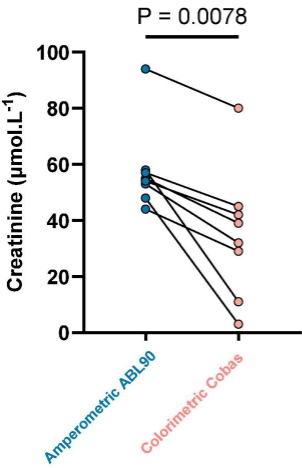
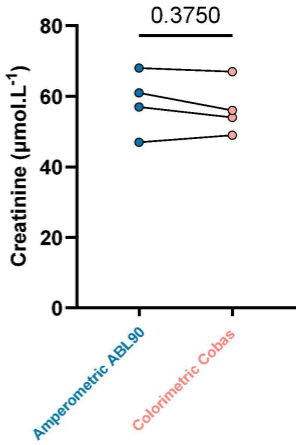


Competitive inhibition

Horseshoe Peroxidase





A NAC-treated**B** Not NAC-treated

- NAC interferes with Trinder-based creatinine assays in a concentration-dependent manner.
- Enzymo-amperometric creatinine assays are unaffected.
- Laboratory Medicine specialists must be aware of this interference and warn clinicians.

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