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Research Article

A Comparative Study about the Effect of Sodium Fluoride and Lithium Heparin on Urea Stability in Human Plasma

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Abstract

Background

Urea is a key marker of renal function, and its measurement may be influenced by preanalytical variables, particularly the choice of anticoagulant.

Methods

This study evaluated urea stability in plasma collected from 325 patients using tubes containing sodium fluoride and lithium heparin. Urea levels were measured at 0, 48, 72, and 96 hours using an enzymatic glutamate dehydrogenase (GLDH) assay on a Thermo Scientific Indiko analyzer. Samples were stored at +4 °C throughout the study period.

Results

A progressive increase in urea concentration was observed in both tube types over 96 hours. For lithium heparin, levels rose from 0.339 ± 0.099 g/L to 0.445 ± 0.112 g/L ($p = 0.002$), and for sodium fluoride, from 0.343 ± 0.145 g/L to 0.460 ± 0.122 g/L ($p = 0.003$). No statistically significant differences were found between the two anticoagulants at any time point ($p > 0.05$).

Conclusions

Both sodium fluoride and lithium heparin maintain acceptable urea assay performance, but refrigeration alone does not fully prevent urea increase over time. Prompt analysis is essential to ensure accurate results.

Keywords: Urea; anticoagulants; preanalytical; variability; biochemistry

Introduction

The accuracy and reliability of results in biology depend primarily on the quality of the pre-analytical phase, with blood sampling being the first crucial step. The most commonly used anticoagulants for blood collection in patients are ethylene diamine tetra acetic acid (EDTA), lithium heparin, sodium citrate, and sodium fluoride [1]. According to World Health Organization recommendations, the choice of anticoagulant not only affects coagulation prevention but can significantly interfere with the measurement of various analytes, particularly those measured by enzymatic methods. Each anticoagulant operates via different mechanisms such as calcium binding or thrombin inhibition and may variably affect analyte stability depending on assay chemistry and sample handling conditions [2]. For many years, manufacturers of medical laboratory automation and reagents have provided guidelines on the types of samples that can be used: plasma and/or serum. They also specify the different types of plasma that are suitable. The most widely recommended anticoagulant for clinical chemistry analyses is lithium heparin [3]. Heparinized plasma offers several advantages, including faster processing and minimal impact on the concentrations of many biochemical markers. However, concerns remain regarding the stability of certain analytes such as urea during storage, especially in samples containing heparin or fluoride.

The influence of anticoagulants used in blood collection tubes for obtaining plasma has been the subject of several studies, considering the recommendations of companies specializing in the production and distribution of laboratory products about interfering anticoagulants. World Health Organization guidelines also highlight that anticoagulants like EDTA, citrate, and fluoride may interfere with enzymatic assays, especially if the metal-ion binding properties or enzyme-inhibitory effects alter the reaction kinetics during analysis [2]. Urea is the primary end product of nitrogen metabolism in humans, formed by the enzymes of the urea cycle, mainly located in the liver. It is then eliminated through bodily fluids such as blood and saliva, particularly via urine [4]. Urea is a polar molecule, highly soluble in water, and neutral in charge, with the chemical formula CH_4N_2O. While blood urea, also known as uremia, varies between 0.130 to 0.450 g/L in men and from 0.130 to 0.400 g/L in women [5]. The clinical significance of urea measurement in blood and serum is well established, especially in the evaluation of renal function and protein catabolism.

Therefore, there is an urgent need to measure urea in biological fluids to diagnose diseases at their early stages. Among the various methods available for urea detection, chromatographic methods are complicated and require time-consuming sample pre-treatment and costly instrumental setups. Biosensing methods overcome these disadvantages as they are simple, quick, specific, highly sensitive, and can also be applied for in vivo urea detection [6]. In addition, enzymatic methods based on urease or GLDH activity are widely used in clinical practice and must be protected from interfering substances introduced during sample collection and storage.

The question arises: which anticoagulant is the best for such assays and ensures the most stable urea measurements over time. This question is particularly important given the need for repeated urea measurements on previously tested samples, especially for patients suffering from renal insufficiency. Temperature has a critical role in analyte stability, where urea remained stable for up to five days at 4°C but degraded rapidly at room temperature [7]. Other findings, suggest that even under refrigerated conditions, urea levels may fluctuate significantly within hours of storage, depending on preanalytical handling and the sample matrix [8]. Generally, serum or plasma is recommended for urea testing. Anticoagulants like fluoride and citrate should not be used due to interference with the analytical method [9]. Nonetheless, sodium fluoride is still used in many clinical settings due to its glycolysis-inhibiting properties, raising questions about its suitability for other assays. World Health Organization further notes that fluoride may inhibit enzyme activity in various assays, which can affect results if proper method validation is not conducted [2]. In this study, we aim to evaluate the effect of sodium fluoride and lithium heparin anticoagulants on the quality of urea results in samples collected in both types of tubes and to compare the stability of these results over a period from 0 to 96 hours when stored at +4°C. This work contributes to the growing body of evidence regarding the impact of anticoagulants on analyte stability and aims to optimize preanalytical protocols for reliable urea assessment in clinical laboratories.

Materials and Methods

The study was carried out between february and march 2023 in Med 2M Laboratory in Tunisia and did not require any additional samples to be taken from patients. We selected patients who had undergone a biochemical check-up in which urea and blood glucose were among the parameters analyzed. To achieve this objective, we conducted a comparative cross-sectional study of 325 patients undergoing routine biochemical monitoring. For each patient, 2 types of tube were collected (vacuette® tube 2 ml lh lithium heparin 13x75 green cap-white ring, premium) and (vacuette® tube 2 ml fx Sodium Fluoride / potassium oxalate 13x75 grey cap-white ring, premium). All tubes were centrifuged for 15 min at 3000 rpm. No hemolysis was detected in any of the samples analysed. The concentration of urea was measured in the 2 tubes containing heparin and fluoride anticoagulants at the same time on the day of sampling, after 48 hours, 72 hours and finally after 96 hours. Throughout these studies the tubes were stored at +4°C. The instrument used was Thermo Scientific™ Indiko™, a fully automated random-loading benchtop analyser for general and specialised biochemistry analyses. Parameters were measured using dedicated techniques and reagents. The principle of the urea concentration measurement procedure is enzymatic. Urea is hydrolysed in the presence of water and urease to give rise to ammonia and carbon dioxide. In the presence of glutamate dehydrogenase (GLDH) and reduced nicotinamide adenine dinucleotide (NADH), ammonia combines with a-ketoglutarate to form L-glutamate. The resulting reduction in absorbance at 340 nm, following the conversion of NADH to NAD, is proportional to the urea concentration in the sample.

Results

The data indicates a consistent and gradual escalation in both heparin and fluoride levels over the 96-hour period. Initially, the measurement of heparin was found to be 0.339 ± 0.099g/l at 0 hours, and its concentration gradually increased to 0.445 ± 0.112g/l by 96 hours (Table1).

Table 1: Urea levels over time using heparin and fluoride anticoagulants.

	Urea measured with anticoagulant	
Time Point	Heparin (mean ± SD) n = 325 (g/l)	Fluoride (mean ± SD) n =325 (g/l)
0 hours	0.339 ± 0.099	0.343 ± 0.145
48 hours	0.357 ± 0.146	0.362 ± 0.146
72 hours	0.362 ± 0.105	0.374 ± 0.122
96 hours	0.445 ± 0.112	0.460 ± 0.122

This increase occurred consistently across the time points, with minor fluctuations observed between the measurements. In a similar manner, the fluoride levels exhibited an initial reading of 0.343 ± 0.145g/l at 0 hours and demonstrated a consistent increase throughout the study, reaching 0.460 ± 0.135g/l at 96 hours (Table1). The increase in fluoride concentration was gradual, with a more moderate rise after 72 hours (Figure 1). It was observed that both anticoagulants exhibited an upward trend, with Heparin and Fluoride concentrations increasing steadily over time (Figure 1).

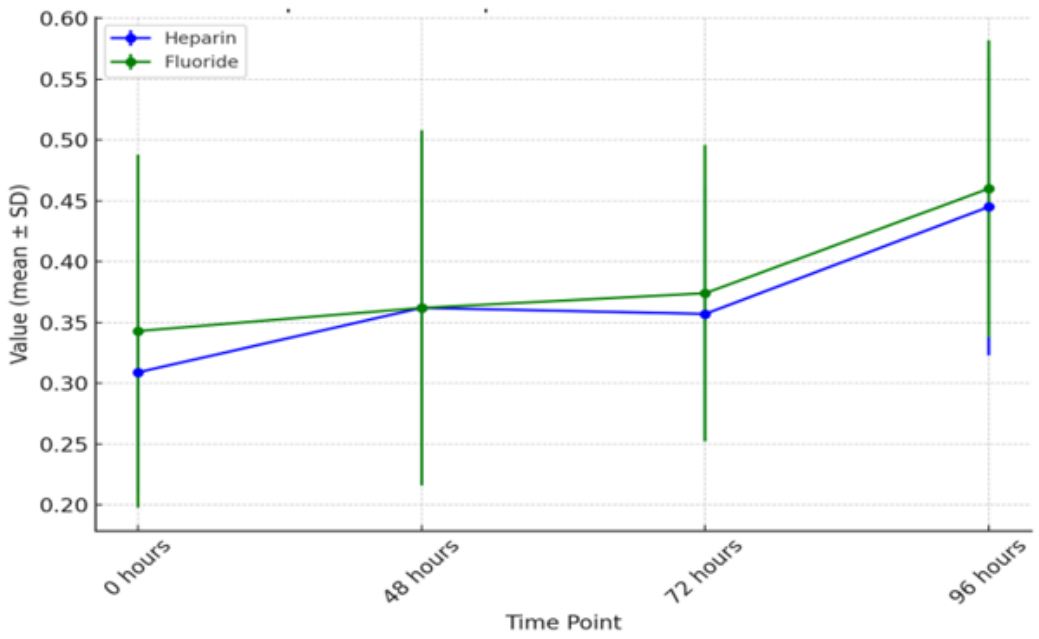

Figure 1: Urea measurements in g/l in tubes containing heparin and fluoride anticoagulants over time.

The data indicates a consistent and gradual escalation in both heparin and fluoride levels over the 96-hour period. Initially, the measurement of heparin was found to be 0.339 ± 0.099g/l at 0 hours, and its concentration gradually increased to 0.445 ± 0.112g/l by 96 hours (Table1). For fluoride, no statistically significant differences were observed between the 0-hour and 48-hour groups (p = 0.635), nor between the 0-hour and 72-hour groups (p = 0.140) (Table 2). However, a statistically significant increase in fluoride levels was observed between 0 hours and 96 hours (p = 0.003), indicating a clear rise in fluoride concentration over the 96-hour period (Table 2). A similar pattern is observed for Heparin, with no significant difference between 0 hours and 48 hours (p = 0.635) or between 0 hours and 72 hours (p = 0.140) (Table 2). However, a significant increase in Heparin levels was observed between 0 hours and 96 hours (p = 0.002), indicating a substantial rise over the study period (Table 2). A comparison of fluoride and heparin at the same time points (0h, 48h, 72h, and 96h) yielded no statistically significant differences, with p-values of 0.635, 0.869, 1.000, and 1.020, respectively (Table 2). The findings indicate that while there is an increase in fluoride and heparin concentrations over time, the levels of these substances do not differ significantly at any of the measured time points.

Table 2: Comparison of urea levels across different anticoagulants and time intervals.

Comparison urea in each anticoagulant	P-value
Fluoride 0h vs 48h	0.635
Fluoride 0h vs 72h	0.14
Fluoride 0h vs 96h	0.003
Heparin 0h vs 48h	0.635
Heparin 0h vs 72h	0.14
Heparin 0h vs 96h	0.002
Fluoride vs Heparin 0h	0.635
Fluoride vs Heparin 48h	0.869
Fluoride vs Heparin 72h	1
Fluoride vs Heparin 96h	1.02

Discussion

The objective of our study was to evaluate the impact of two commonly used anticoagulants, sodium fluoride and lithium heparin, on the stability of urea measurements over time when samples are stored at +4°C. We observed a progressive increase in urea concentrations in both anticoagulant tubes over 96 hours, with levels significantly elevated at 96 hours compared to baseline ($p = 0.003$ for fluoride, $p = 0.002$ for heparin). These findings suggest that urea concentrations may become artificially elevated over time, even under refrigerated conditions. This observation contrasts with findings reported by [7], who observed a decrease in urea concentration after 24 hours when samples were stored at room temperature (32°C), while urea remained stable for up to 5 days when refrigerated at 4°C. Their study highlighted the critical role of temperature in analyte stability, with refrigeration significantly preserving urea levels, though their results showed degradation rather than an increase in urea [7]. Similarly,[8] demonstrated that urea concentrations in serum samples stored at 4°C showed a slight increase at 4 hours but decreased significantly after 8 and 12 hours, with further decline after prolonged storage at -20°C [8]. The progressive increase observed in our samples suggests possible interference related to anticoagulants or preanalytical factors differing from those in previous studies.

Additionally [10] reported no significant change in urea levels for up to 72 hours at 2–8°C in uncentrifuged samples, though differences in storage and sample handling may explain variation in results [10]. The increasing urea concentrations in our samples despite refrigeration could indicate biochemical or enzymatic reactions influenced by sodium fluoride and lithium heparin anticoagulants, potentially affecting urea assay accuracy. These findings emphasize the importance of anticoagulant choice and storage duration on urea stability. The progressive increase in urea concentrations suggests that sodium fluoride and lithium heparin tubes may not fully preserve urea stability over 96 hours at +4°C, highlighting the necessity for timely analysis. Further research on the mechanisms causing this increase and evaluation of alternative anticoagulants or protocols are warranted. Our study found no statistically significant difference in urea concentrations measured simultaneously in fluorinated (sodium fluoride) and heparinized tubes ($p > 0.05$), indicating that these two anticoagulants may exert a comparable impact on urea stability during storage at +4°C over 96 hours. This finding aligns well with the observations of [11], who reported that fluoride and heparin had a negligible effect on urea levels, although minor variations were noted depending on other biochemical parameters [11]. Interestingly, the lack of a differentiated effect between fluoride and heparin resonates with previous recommendations cautioning against the use of fluoride in certain enzymatic assays due to potential interference [2,7]. However, our results suggest that the enzymatic urea assay based on glutamate dehydrogenase (GLDH) remains stable and unaffected in the presence of fluoride, underscoring the robustness of this particular measurement protocol under varying anticoagulant conditions [8].

One limitation of our study is the absence of a serum control group, which would have allowed a more comprehensive comparison of anticoagulant effects versus natural clotting on urea stability. Serum measurements could clarify whether anticoagulants introduce subtle biases or preserve analyte integrity better. Furthermore [12] highlight that urea stability may depend significantly on the analytical instrument and method used; as our study employed a single device and enzymatic test, the generalizability of results across different platforms remains to be assessed. Future studies would benefit from expanding the scope to include other routine biochemical analytes, comparing plasma versus serum samples, and evaluating stability at room temperature to better simulate typical clinical workflows. Additionally, extending the duration beyond 96 hours could help establish definitive guidelines for maximum sample shelf-life, ensuring reliable urea determinations in various healthcare settings [7,10]. Our study demonstrates that urea concentrations progressively increase over 96 hours of storage at +4°C in both sodium fluoride and lithium heparin anticoagulant tubes. This rise contrasts with previous reports suggesting stability or even a decline in urea levels under similar or varied storage conditions. The observed discrepancy indicates that the choice of anticoagulant and preanalytical

handling may significantly impact urea assay accuracy, even under refrigeration. Overall, our findings contribute valuable evidence supporting the equivalency of sodium fluoride and lithium heparin anticoagulants for urea testing when using a GLDH enzymatic assay at refrigerated conditions, emphasizing the importance of method-specific validation in clinical biochemistry.

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Conflict of Interest Statement

The authors declare no conflicts of interest.

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