Urine

Effects of varied temperature and time exposures on the stability of creatinine, iodine and total protein in urine samples

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To,

The Editor

Urine

Subject: Submission of manuscript for publication

Sir,

We are submitting the manuscript titled “Effects of varied temperature and time exposures on the stability of creatinine, iodine and total protein in urine samples” to your esteemed journal for publication.

The stability of biochemical indicators when sample processing or analysis is delayed, particularly in large scale surveys, has been subject to discussion and has been a reason for not including such objective measures in population surveys. However, knowledge of the impact of such factors such as ‘time delays’ and ‘high temperature’ will help to improve pre-analytical phase of field surveys. This study reports the effects of different temperature/time exposures likely on urinary creatinine, iodine, and total protein levels .

This paper may be considered under ‘ Original article’ category. This paper has not been published in any format earlier. All the authors approved the final version of the manuscript and agree to be accountable for all aspects of the work. The manuscript has a word count of 1800, one table and four figures.

Thanking you

Yours Sincerely

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Effects of varied temperature and time exposures on the stability of creatinine, iodine and total protein in urine samples

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Abstract

Delayed processing of biological samples is common in largescale field surveys, which may affect the stability of analytes. A cross-sectional study (N=35) was conducted to examine the effects of 10 different temperature-time exposures, likely to be encountered in survey settings, on urinary analytes compared to a respective reference value (T_0). Mean percentage differences between reference and test concentrations were compared against the calculated total change limit (TCL), and were found to be within the TCL. The findings suggest that reliable measurements can be obtained from unpreserved urine up to 24 hours when immediate processing of samples is not feasible.

Keywords: stability, preanalytical, temperature, urinary, analytes

1.Introduction

Blood and urine samples collected in large-scale field surveys are commonly used to assess and monitor a population's nutritional status. Assessment of biomarkers provides valuable information on health exposures, susceptibility to diseases, and response to intervention. However, delays in sample processing in resource-poor settings may subject samples to adverse environmental conditions for varied lengths of time, affecting the stability of analytes and the accuracy of measurement indicators. Therefore, knowledge of the impact of such factors on the stability of analytes is important to improve quality during the pre-analytical phase of field surveys.

Previously, we have reported that many nutritional indicators, other than creatinine, folate and hemoglobin, can be reliably estimated in blood samples exposed to 22-30°C for 12 hours [1]. However, little is known about the effects of different temperature/time exposures likely to be encountered in large-scale field surveys on urinary creatinine, iodine, and total protein levels which were examined in this study.

2. Materials and methods

This was a cross-sectional study conducted during December 2017 to January 2018 in Delhi, that involved comparing 10 different temperature and time exposures against a reference condition. A sample of 35 was required to detect a mean percentage difference of 0.5 between two groups with 80% power and $\alpha = 0.05$, based on information from prior literature [2, 3]. Individuals $\geq$ 18 years of age were recruited through a camp-based approach in Delhi. The study was approved by the Ethics Committee, All India Institute of Medical Sciences, New Delhi. Written informed consent was obtained from all individuals prior to study participation.

2.1 Sample collection

Participants were instructed to collect a clean-catch first morning voided urine sample (~100 ml) in a provided sterile container. Urine samples were aliquoted into different sterile capped storage tubes for further analysis. One aliquot of urine was transferred immediately to a sterile tube and stored at 2-8°C considered the reference sample for measuring creatinine, iodine and total protein. The other aliquots were tightly capped and were exposed to 2-8°C for 6 hrs, room temperature (RT) for 6, 12 and 24 hrs, 22-30°C for 6, 12 and 24 hrs and >30°C for 6, 12 and 24 hrs (Figure 1). For maintaining 2-8^{o}C, samples were stored with ice packs in thermacol boxes. For 22-30°C and >30°C, hot water bags were placed in thermacol boxes, over layered with a cloth towel over which thermacol stands with samples were kept. Data loggers (tempmateM1®, Germany) inside the thermacol boxes monitored and recorded real-time temperatures and changes in conditions over time and recorded temperature. between 3-6^{o}C for samples kept in ice packs, 17-19^{o}C for samples kept at room temperature, 24-26^{o}C for samples kept at 22-30^{o}C and 38-40^{o}C for samples kept at >30^{o}C. Once in the laboratory, samples were transferred to incubators to maintain the respective temperatures.

Biochemical analysis**:** Urine samples were analysed for iodine by manual method (based on the Sandell-Kolthoff reaction) [4]. Total protein (spectrophotometric, biuret method) and creatinine (spectrophotometric, alkaline picrate kinetics, Jaffe's method) were measured on the analyser (Beckman Coulter, AU 680, USA). To lessen the run to run variability, samples were processed in the same batch. The analytical coefficient of variation (CVa) was computed using average of laboratory quality control samples over 2 months while the biological coefficient of variation (CVb) for creatinine and protein (CV b) were adopted from Westgard's desirable biological

variation database specifications [5]. The CVb for iodine was adopted from Andersen (2009) et al. [6]. The AIIMS laboratories participates in RIQAS (RANDOX external quality assurance scheme) EQAS program for urinary protein and creatinine and EQUIP (Ensuring The Quality Of Iodine Procedures) a standardization programme of CDC, Atlanta for urinary iodine.

2.2 Statistical analysis

The percentage deviation in the analyte value was computed by subtracting values at baseline (To) from that at other exposure (Tx) using the following formulae:

Percentage deviation = $[(Tx - To)/To] \times 100$

For assessing changes resulting from instability of an analyte, in the same sample from the same individual, analytical variance or coefficient of variation (CVa) and biological coefficient of variation (CVb) (within- subject biological variation) for each biological indicator was taken into consideration. As in this study, the specimens were collected only single time and then aliquoted in different tubes, thus the biological variation could not be estimated from this study data and therefore CVb for each analyte was taken from the literature of biological variation for analytes [5,6]. The analytical and intra-individual imprecision were used to estimate the total change limit (TCL) for each analyte in the following manner [3].

$$TCL = \sqrt{(2.77\ CVa)^2 + (0.5\ CVb)^2}$$

The factor 2.77 is derived from $Z\sqrt{2}$, where $Z=1.96$, determined by the 95% confidence interval value for bi-directional changes. According to the recommendations of the College of American Pathologists [7, 8], the imprecision of a method, for individual single and multipoint testing, should be equal or less than one-half of the average within-subject variation (CVb), and this

should be the goal for short-term laboratory imprecision (≤0.5 CVb). Table 1 presents the analytical and biological CV% for the different analytes and the computed TCL values.

The mean % deviation of the analytes at each temperature and duration of exposure was compared with the TCL. Values that fell within the TCL were deemed to be stable. In cases where the results for an analyte had a mean percentage difference that exceeded the TCL, then the difference was judged to be significant and not to meet the stability criteria. All statistical analysis was performed using STATA 15.1 (STATAcorp LP, College station, TX 77845, USA).

3. Results

A total of 35 participants aged ≥ 18 years were recruited. Analytical coefficient of variation, within subject biological variation and computed total change limit for the selected urinary analytes was shown in Table 1. The TCL values for urinary creatinine, urinary protein and urinary iodine are 27.9, 25.4 and 14.6 respectively. Table 2 shoes the mean values for creatinine, iodine and protein at 10 different exposure conditions.

Figure 2,3 & 4 shows the mean % deviation for urinary creatinine, protein and iodine, from reference values, in urine samples exposed to different temperatures and centrifugation time. As evident from the figures mean % deviation for the analyte studied were found to be within the TCL limits at all temperatures and time periods studied. In addition, there were no noticeable trends in analyte concentrations as a function of time and/or temperature.

4. Discussion

In this study, we examined the stability of creatinine, iodine and total protein in urine samples subjected to various temperature and time exposures likely to be encountered in a survey setting. Individual and mean percentage differences for each analyte were within respective total change limits, suggesting that creatinine, iodine, and total protein concentrations were stable at all temperature-time points studied. This is consistent with other studies that showed…---------…(?) and suggests that, compared to serum, urine has a higher thermodynamic stability, fewer protein interactions and a lower protein complexity [7, 8].

Urinary creatinine has shown both short-term and long-term stability across temperature/time exposures and is, therefore, often used as a reference parameter for other analytes. Spierto et al examined the stability of urinary creatinine at 4°C, 25°C, 37°C and 55°C up to 30 days using a two-point enzymatic creatinine test and found no significant change in creatinine concentrations after storage at 4°C up to 30 days and at 25°C up to 8 h. [8]. However, decreases in creatinine concentrations were observed at higher temperatures (~ 10% decrease at 37°C up to 4 days; ~15% decrease at 37°C up to 10 days; and 3% decrease at 55°C up to 2 days) [8]. Miki et al observed stable urinary creatinine concentrations following storage at 4°C for one week under pH 0.5-7 [11]. A study conducted by Parekh et al [12] demonstrated long-term stability of urinary creatinine at -70°C for up to ~2.5 years using the picric acid colorimetric method. Remer et al [13] observed stable urinary creatinine concentrations in samples from the German DONALD study stored at -22°C for 15 years using the Jaffe method. Chapman et al demonstrated a small and clinically non-significant reduction in urinary creatinine in samples stored at -20°C and at -80°C for 12 hours, also using the Jaffe method. [14]. A limitation of our study is that we assayed creatinine in urine samples only using the Jaffe method which is

susceptible to the presence of ketones and pyruvate over a longer sample storage duration. Therefore, sample stability may be affected with storage at higher temperatures for > 24 hours.

We observed stable total protein concentrations at all temperature-time points examined. A study conducted by Ercan et al [15] that examined the effects of short-term refrigeration on urinary protein showed 100% concordance at 4 hours and 97.9% concordance at 8 hours, compared to optimal conditions. In contrast, Berkel et al [16] observed a 49% increase in protein concentration in urine samples stored at room temperature for 72 hours and assayed using the biuret method. We also observed stable urinary iodine concentrations at all temperature-time exposures in our study. Few studies have investigated the effect of temperature and time duration on the stability of urinary iodine and reported varied results. Blazewicz et al [17] observed a 14% decrease in urinary iodine after storage at -25°C for 24 weeks when assayed by ion chromatography with pulsed amperometric detection Remer et al [13] examined the long-term stability of iodine after storage at -22°C for 15 years and observed a 4% increase when 24-hour urinary iodine was estimated using a modified Sandell-Kolthoff reaction. The Sandell-Kolthoff method, which we used in our study, is sensitive to temperature and may induce a rapid reduction of ceric to cerous ion at higher temperatures, possibly affecting the stability of iodine [18].

Our study strengths include assessing the effects of multiple temperature-time exposures likely to be encountered in field-based epidemiological surveys. The first voided morning urine sample appeared to be a simple and reliable collection method, as compared to more complex 24-hour and spot urine samples. Further, urine collection in plain sterile storage vials reduced the potential interference of additives in the urine analysis, which could have occurred when using

pre-treated storage vials. A limitation of our study is that we could not assess concentrations of creatinine, iodine and total protein under longer storage conditions.

In conclusion, our study revealed that high temperature exposures up to 24 hours had no substantial effect on the stability of urinary creatinine, iodine and total protein concentrations. These results suggest that reliable measurements can be obtained from unpreserved urine up to 24 hours when immediate processing of samples is not feasible.

5. Funding Source

This study was conducted as part of the 2016-2018 Comprehensive National Nutrition Survey in India that was funded by Aditya Mittal, President of ArcelorMittal, and Megha Mittal, Managing Director of ESCADA.

6. Conflicts of interest

The authors declare that they have no conflicts of interest. The views expressed are those of the authors and do not necessarily reflect their respective organizations.

7. Acknowledgments

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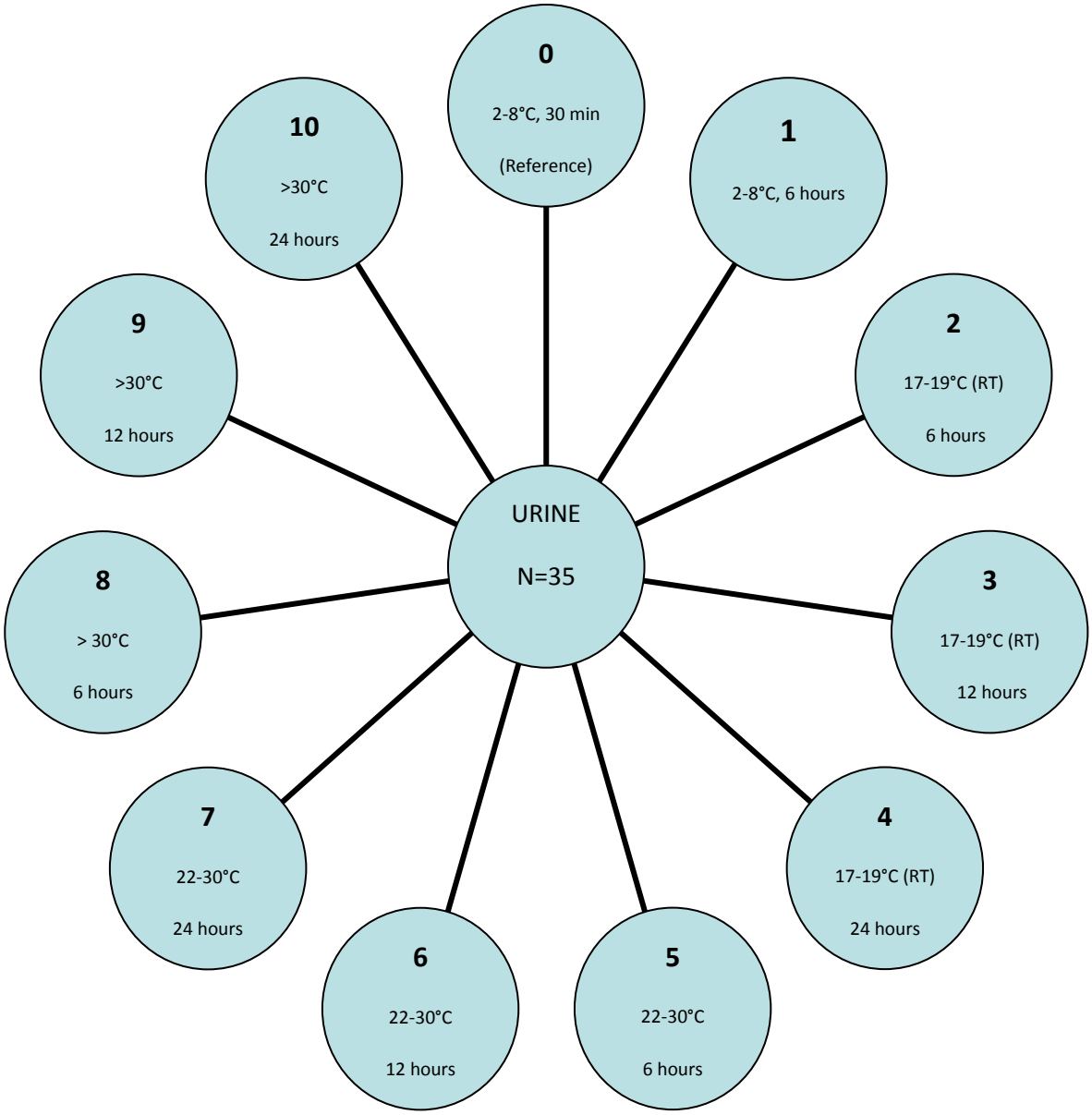
Table 1: Analytical coefficient of variation, biological coefficient of variation, and total change limit for urinary creatinine, iodine, and total protein

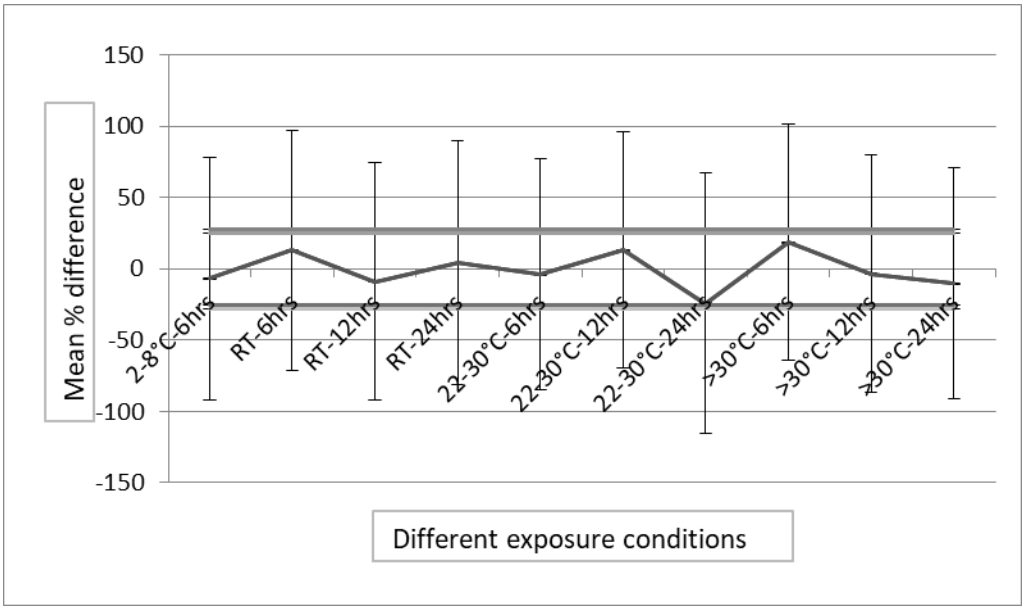
Analyte	Analytical coefficient of variation (CVa) %	Within-subject biological coefficient of variation (CVb) %	Total change limit (TCL) %
Creatinine	7.7	36.3	27.9
Iodine	3.0	24.0	14.6
Total protein	6.5	35.5	25.4

Figure 1: Temperature and time exposures for urine samples



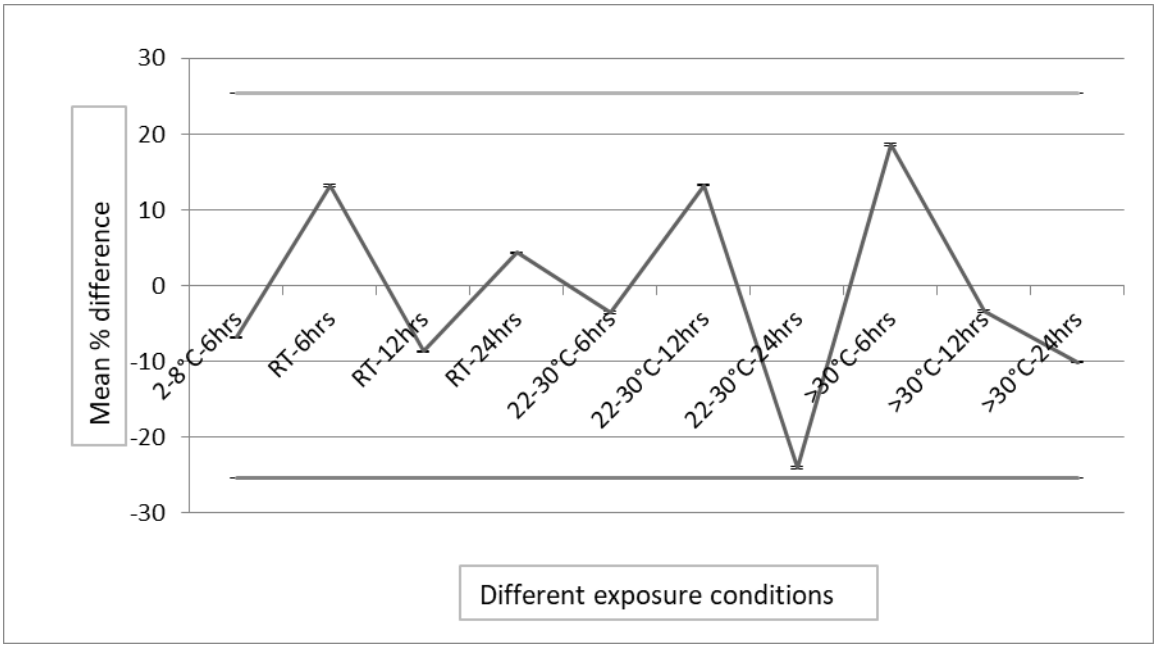
Legend to figure 1: The collected Urine was aliquoted and were exposed to 10 different temperatures and time. After exposure the analytes were measured.

Figure 2: Percentage differences in urinary creatinine concentration between reference and comparison temperature-time exposures



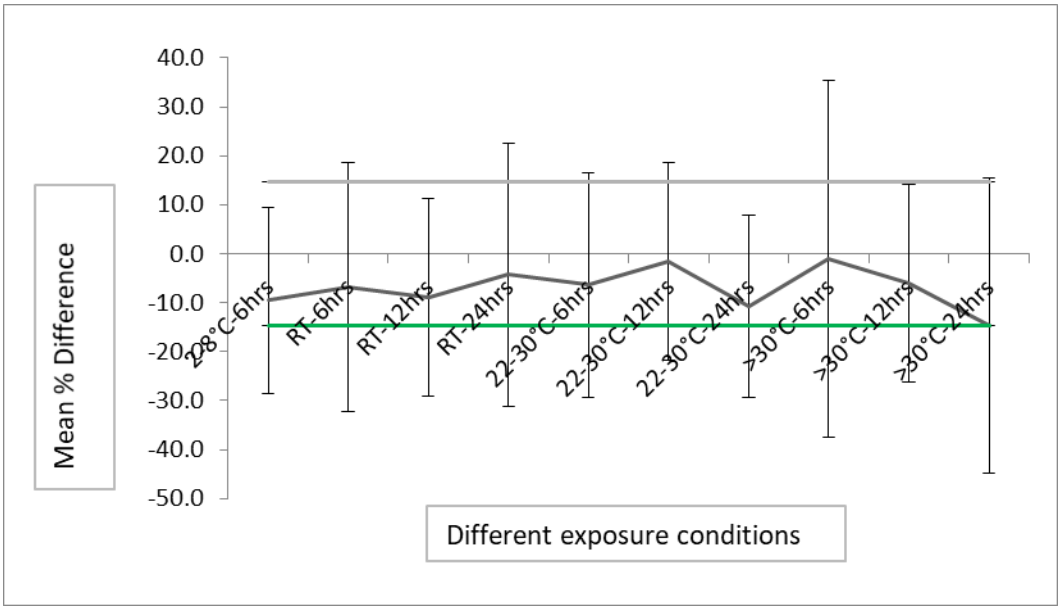
Legend for figure 2: Figure shows the mean % deviation of creatinine from baseline values in urine samples exposed to different temperatures (2-8^0 C, RT, 22-30^{0}C and >30^{0}C) for varying lengths of time (6 h, 12 h and 24 h) . The horizontal lines above and below the mean indicate the TCL value (±27.9)

Figure 3: Percentage differences in urinary total protein concentration between reference and comparison temperature-time exposures



Legend for figure 3: Figure shows the mean % deviation of total protein from baseline values in urine samples exposed to different temperatures (2-8^0 C, RT, 22-30^{0}C and >30^{0}C) for varying lengths of time (6 h, 12 h and 24 h). The horizontal lines above and below the mean indicate the TCL value (±25.4)

Figure 4: Percentage differences in urinary iodine concentration between reference and comparison temperature-time exposures



Legend for figure 4: Figure shows the mean % deviation of urinary iodine from baseline values in urine samples exposed to different temperatures (2-8^0 C, RT, 22-30^{0}C and >30^{0}C) for varying lengths of time (6 h, 12 h and 24 h) prior. The horizontal lines above and below the mean indicate the TCL value (±14.6)

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Conflicts of interest

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