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Can we reduce the interference of vitamin C and PH in urinalysis?

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Abstract

Urinalysis is often interfered by vitamin C and PH. This study was to evaluate the interference of vitamin C and PH in urinalysis, and minimize the risk to make the effective communication. 732 leftover urine samples in which the concentration of vitamin C was 0.0 mmol/L and either of the test-strip results (Glucose, Blood, Bilirubin, Leucocyte, Nitrite, Protein, Ketone) was positive that were collected. The interference test checked out using these leftover urine samples. When the chemical protein module was positive, the sulfosalicylic acid test performed to confirm the positive results. About 27% of urine samples were vitamin C positive. Adequate vitamin C would interfere with the chemical result of GLU, BIL, and BLD, but not KET, PRO, WBC, NIT (p-value < 0.05). False-positive protein occurred at any samples, but higher when urine PH is greater than or equal to 8.0. In summary, to minimize the risk of vitamin C and PH in urinalysis, the laboratory should pay more attention to its conditions. Also, do more to help guarantee the accuracy of the urinalysis results and to give more comments to doctors to achieve effective communication.

Keywords: interference; PH; urine strip test; vitamin C.

Practical Application: Helping to analyze the effect of Vitamin C and food taken on the urinals, and improve people's health life.

1 Introduction

As we know, urine specimens are usually readily available and obtain non-invasive (Liao & Churchill, 2001). And urine tests are often used to help doctors to diagnose and evaluate diseases. So, urinalysis is useful as a screening test and becomes an essential part of clinical assessment. Unfortunately, chemical tests are often interfered with by lots of factors, such as vitamin C and PH (Unic et al., 2018). To provide accurate reports to doctors, laboratory scientists should recognize and reduce false positive or false negative results (Poloni et al., 2018). In our department, amounts of DIRUI dipsticks (FUS-12MA II) consume every day. Whether PH and vitamin C have an impact on the test results of DIRUI dipsticks? If the answer is definitely, how can we minimize or eliminate interference? To minimize these risks, can we establish some review rulers or give some comments?

2 Materials and methods

2.1 Reagents and equipment

Use FUS-3000plus, Dirui Industrial Co., Ltd hybrid analyzer, and DIRUI dipsticks (FUS-12MA II) to evaluate interference according to the Dirui protocol guidelines (Dirui Guidance Standard for Customers). Before starting these tests, FUS-3000plus was calibrated according to the manufacturer's recommendations. Quality control was performed using the matching chemical analysis quality control (Dirui Industrial Co., Ltd).

2.2 Test for vitamin C in urine

Leftover urine from patient specimens from outpatient was ready for use. Seven hundred thirty-two urine samples in which the concentration of vitamin C was 0.0 mmol/L and either of the test-strip results (Glucose, Blood, Bilirubin, Leucocyte, Nitrite, Protein, Ketone) was positive that were chosen.

2.3 Preparation before interference test

Vitamin C 100 g/L aqueous solution, dissolve 10 g of vitamin C in pure water to make 100 mL. Sulfosalicylic acid (SSA) 200 g/L aqueous solution, dissolve 20 g of sulfosalicylic acid in pure water to make 100 mL.

2.4 Interference test

1, 3, 5 or 10 µl vitamin C100 g/L aqueous solution per mL urine was added respectively into these chosen urine samples to achieve Vitamin C 0.6,1.4,2.8,5.7 mmol/L and retested by FUS3000plus.

If the protein's module was positive, the sulfosalicylic acid test performed to confirm the positive results. One mL of the urine samples placed into a 5 mL transparent glass tube and two drips of SSA added. The degree of protein precipitation classified as the following grade: no increase in turbidity was labeled Negative. When the following situation occurred, we consider it as a true-positive result. (a) barely perceptible

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turbidity; (b) distinct turbidity but not discrete granulation; (c) turbidity with granulation but no flocculation; (d) turbidity with granulation and flocculation; (e) clumps of precipitated protein, or solid precipitate.

2.5 Statistics

The statistical analysis was carried out using SPSS version 24.0. A Chi-square test used for statistical analysis. According to the comparison rules of chemical urinalysis, positive results cannot be negative, and the grading bias does not exceed one grade. If the positive results become negative, or the grading bias exceeds one degree, the repeat-ability of urinalysis instrument is poor. The rate of instrument repeat-ability should be greater or equal to 80%. P-value < 0.05 was considered to indicate statistical significance.

3 Results and discussion

3.1 The concentration of vitamin C in urine

During 2019 November 1st and 2020 January 31st, these chemical results of 34,279 urine samples were retrospectives, of which 27.7% observed vitamin C positive (Table 1).

3.2 Vitamin C added in urine and its interference to urinalysis results

When Leftover urine samples added vitamin C 100 g/L aqueous solution and reached the concentration of urine vitamin C was 5.7 mmol/L, it would interfere the chemical result of GLU, BIL, and BLD, but not KET, PRO, WBC, NIT, p-value < 0.05 (Table 2).

When DIRUI dipsticks showed the concentration of vitamin C 2.8 mmol/L, it would interfere with the chemical result of GLU and BLD, p-value < 0.05 (Table 3). When DIRUI dipsticks showed the concentration of vitamin C, either 1.4 mmol/L or 0.6 mmol/L, it would only interfere with the chemical result of BLD, p-value < 0.05 (Tables 4 and 5). When urine chemical results of GLU, BIL, and BLD have lower grades, they are easier to interfere with by higher concentration vitamin C.

3.3 Results of protein in urine samples

Among 626 urine samples with a protein module positive, the observed pH of 520 urine samples was greater than or equal to 8.0, while the observed pH of others was < 8.0. SSA tests (Figure 1) were performed to validate these positive results of the protein module. Our data showed that false-positive protein occurred at any samples, but higher when urine PH is greater than or equal to 8.0. Chi-square were performed, p-value < 0.05 (Table 6).

Table 1. The concentration of vitamin C in urine.

Concentration of vitamin C (mmol/L)	0.0	0.6	1.4	2.8	≥ 5.7
number of samples	24782	10257	3103	1642	1345

Table 2. Vitamin C was added to obtain its concentrations (≥ 5.7 mmol/L) in urine and its interference to urinalysis results.

Concentration gradient		Concentration of vitamin C (mmol/L)		NO.	Difference
		0.0	≥ 5.7		
Glucose (GLU) (mmol/L)	0 (-)	±	-	39*	2
	2.8 (±)	±	±	3*	0
	5.6 (+)	+	-	15	2
	5.6 (+)	+	±	4	1
	14 (2 +)	2 +	-	9	3
	14 (2 +)	2 +	±	11	2
	14 (2 +)	2 +	+	14	1
	14 (2 +)	2 +	2 +	9	0
	28 (3 +)	3 +	2 +	15	1
	28 (3 +)	3 +	3 +	5	0
Total				74*	≥2
				54 *	<2
Ketone (KET) (mol/L)	0 (-)	±	-	4	2
	0.5 (±)	±	±	17	0
	1.5 (+)	+	+	3	0
	3.9 (2 +)	2 +	+	5	1
	3.9 (2 +)	2 +	2 +	2	0
	7.8 (3 +)	3 +	3 +	2	0
	7.8 (3 +)	4 +	4 +	2	0
Total				4#	≥ 2
				31#	< 2
Protein (PRO) (g/L)	0 (-)	±	-	4	2
	0.15 (±)	±	±	17	0
Total				4#	≥ 2
				17#	< 2
Leucocyte (WBC) (cells/μL)	0 (-)	±	-	23#	2
	15(±)	±	±	53#	0
	70 (+)	+	±	8	1
	70 (+)	+	+	6	0
	125 (2 +)	2 +	+	4	1
	125 (2 +)	2 +	2 +	11	0
	500 (3 +)	3 +	2 +	1	0
	500 (3 +)	3 +	3 +	17	0
Total				23#	≥ 2
				100#	< 2
Nitrite (NIT) (mg/dL)	negative (-)	positive	negative	6	2
	positive (+)	positive	negative	9	0
Total				6#	≥ 2
				9#	< 2
Bilirubin (BIL) (μmol/L)	0 (-)	+	-	28	2
	17 (+)	+	+	5	0
Total				28*	≥ 2
				5*	< 2
Blood (BLD) (cells/μL)	0 (-)	±	-	39*	2
	15 (±)	±	±	11*	0
	25 (+)	+	-	21	2
	25 (+)	+	±	10	1
	80 (2 +)	2 +	±	6	2
	80 (2 +)	2 +	+	5	1
	200 (3 +)	3 +	+	3	2
	200 (3 +)	3 +	2 +	10	1
	200 (3 +)	3 +	3 +	13	0
Total				69*	≥ 2
				49*	< 2

Base on the comparison rules of chemical urinalysis, positive results cannot be negative, and the grading bias does not exceed one grade. If the positive results become negative, or the grading bias exceeds one grade, the difference is categorized to ≧ 2. The rate of instrument repeat-ability is calculated at 80%; *Chi-square were performed, p-value < 0.05; # Chi-square were performed, p-value > 0.05.

Table 3. Vitamin C was added to obtain its concentrations (2.8 mmol/L) in urine and its interference to urinalysis results.

Concentration gradient		Concentration of vitamin C (mmol/L)		NO.	Difference
		0.0	2.8		
GLU (mmol/L)	0 (-)	±	-	56*	2
	100 (±)	±	±	12*	0
	250 (+)	+	-	3	2
	250 (+)	+	±	14	1
	500 (2 +)	2 +	±	3	2
	500 (2 +)	2 +	+	3	1
	500 (2 +)	2 +	2 +	4	0
	2000 (3 +)	3 +	2 +	2	1
	2000 (3 +)	3 +	3 +	4	0
Total				62*	≥ 2
				39*	< 2
BIL (mg/dL)	0 (-)	+	-	3	2
	17 (±)	+	+	7	0
Total				3#	≥2
				7#	<2
BLD (cells/μL)	0 (-)	±	-	51*	2
	10 (±)	±	±	12*	0
	25 (+)	+	+	1	0
	80 (2 +)	2 +	±	7	2
	200 (3 +)	2 +	+	13	1
	200 (3 +)	2 +	2 +	4	0
	200 (3 +)	3 +	3 +	4	0
Total				58*	≥ 2
				34*	< 2

Base on the comparison rules of chemical urinalysis, positive results cannot be negative, and the grading bias does not exceed one grade. If the positive results become negative, or the grading bias exceeds one grade, the difference is categorized to ≧2. The rate of instrument repeat-ability is calculated at 80%; *Chi-square were performed, p-value < 0.05; # Chi-square were performed, p-value > 0.05.

Table 4. Vitamin C was added to obtain its concentrations (1.4 mmol/L) in urine and its interference to urinalysis results.

Concentration gradient		Concentration of vitamin C (mmol/L)		NO.	Difference
		0.0	1.4		
GLU (mmol/L)	0 (-)	±	-	9	2
	2.8 (±)	±	±	12	0
	5.6 (+)	+	±	2	1
	5.6 (+)	+	+	3	0
Total				9#	≥ 2
				17#	< 2
BLD (cells/μL)	0 (-)	±	-	9	2
	10 (±)	±	±	3	0
Total				9*	≥ 2
				3*	< 2

Base on the comparison rules of chemical urinalysis, positive results cannot be negative, and the grading bias does not exceed one grade. If the positive results become negative, or the grading bias exceeds one grade, the difference is categorized to ≧2. The rate of instrument repeat-ability is calculated at 80%; *Chi-square were performed, p-value < 0.05; #Chi-square were performed, p-value > 0.05.

Table 5. Vitamin C was added to obtain its concentrations (0.6 mmol/L) in urine and its interference to urinalysis results.

Concentration gradient		Concentration of vitamin C (mmol/L)		NO.	Difference
		0.0	0.6		
BLD (cells/μL)	0 (-)	±	-	15	2
	10 (±)	±	±	3	0
Total				15*	≥ 2
				3*	< 2

Base on the comparison rules of chemical urinalysis, positive results cannot be negative, and the grading bias does not exceed one grade. If the positive results become negative, or the grading bias exceeds one grade, the difference is categorized to ≧2. The rate of instrument repeat-ability is calculated at 80%; *Chi-square were performed, p-value < 0.05; #Chi-square were performed, p-value > 0.05.

Table 6. Results of protein in urine samples.

PH	the number of samples		
	DIRUI dipsticks	SSA positive[1]	SSA negative[1]
	Positive results of protein		
PH ≥ 8.0	520	56	464*
PH < 8.0	106	101	5*

[1]200 g/L SSA test was performed to confirm the result of protein; *Chi-square were performed, p-value < 0.05.

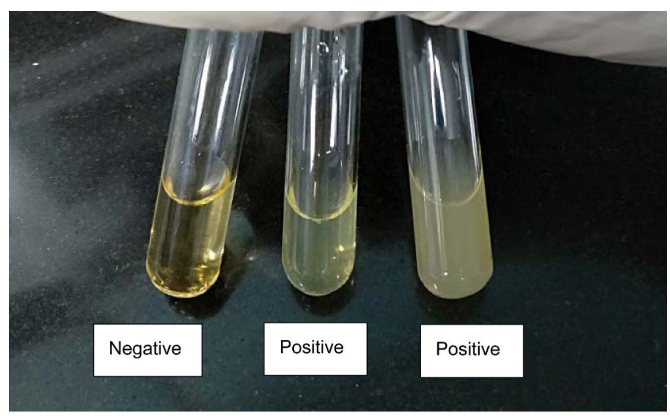

Figure 1. Results of protein with the method of SSA tests.

3.4 Discussion

Ascorbic acid or vitamin C, which is found widely in many fruits and vegetables, is an essential water-soluble micro-nutrient involved in many synthesis mechanisms in our body. For example, it plays a significant role in defense against increased oxidative stress during pregnancy (Ibrahim et al., 2016). Though taking vitamin C supplements during pregnancy does not help prevent pregnancy problems, including stillbirth, the death of the baby, preterm birth, pre-eclampsia, or low birth weight babies (Rumbold et al., 2015). But maternal vitamin C intake was positively associated with the abdominal circumference of the fetus and infant birth length (Jang et al., 2018). In our hospital, pregnant women are suggested to take the multivitamin supplement containing vitamin C (Elevit Pronatal, Bayer S.A.).

Consumption of fruits and vegetables often recommend because of their excellent nutrient profile, high dietary fiber content, and generally low caloric content (Wagner et al., 2016). Diets rich in fruit and vegetables have linked with a reduced risk of chronic disease (Woodside et al., 2017). For healthy, pregnant women need to consume amounts of fruits and vegetables every day, which happen to contain lots of vitamin C. That's why our data showed that the incidence of urinary vitamin C was 27.7% of 34,279 urine samples, which was higher than 18.1% of 5,006 routine urinalysis specimens (Lee et al., 2017). Vitamin C's impact in chemical tests is widespread, not only in urine chemical tests but also in serum biochemical tests. Martinello F showed in the presence of high concentrations of vitamin C negative interference that occurred, particularly on the measurement of cholesterol and glucose (Martinello & Silva, 2006). In this study, almost all of the urine samples were collected from pregnant women whose urine contains amounts of vitamin C. So, in our department, the negative interference of vitamin C is not easy to avoid.

According to CNAS-CL02-A002, the comparison rules of chemical urinalysis was that positive results could not be negative, and the grading bias does not exceed one grade. When positive results became negative, and the grading bias exceeded one grade, the results were considered as effective interference. Impact factors cause not all of the false-negative results, and sometimes errors can happen. Here we allowed 20% errors. The rate of instrument repeat-ability was calculated at 80%. We agreed with that high vitamin C concentration possibly can cause false-negative urinary glucose test results (Ritterath et al., 2006). Our data showed that when the concentration of urine vitamin C was 5.7 mmol/L, it would interfere with the chemical result of GLU, BIL, and BLD, but not KET, PRO, WBC, NIT. Though Wonmok Lee etc. (Lee et al., 2017) showed that vitamin C interferes with hemoglobin and leukocyte esterase results. Vitamin C can cause the urine dipstick result was two or more grades lower than the urine sediment result. But they could not exclude the fact that some WBC are lymphocytes and some RBCs are unbroken, or maybe other factors cause the false-negative, such as time and centrifuge (Becker et al., 2016; Dolscheid-Pommerich et al., 2016). Nitrite tests gave false-negative results at lower concentrations of vitamin C (Lee et al., 2017). Unfortunately, here our data could not support it. Sometimes, urinary tract infection does not cause urine nitrite results positively (Schroeder et al., 2015), so only 15 urine samples in which nitrite results were true-positive collected. And then DIRUI Urine Analysis Test Strip has higher limits of detection at nitrite. Only when nitrite ≥ 0.125 mg/L, DIRUI Urine Analysis Test Strip would show nitrite positive.

As we know, proteinuria plays an important role in the diagnosis, treatment, and prognosis of renal diseases such as nephrotic syndrome or end-stage renal disease. Meanwhile, the duration of proteinuria may be more important than quantity in the clinical assessment (Liao & Churchill, 2001; Usui et al., 2018). According to the DIRUI Urine Analysis Test Strip Instructions, the PH module determine by indicator protein error. which means the negative charge of a protein-based on a specific PH indicator attracts by the cations of the protein and further ionizes to cause the indicator to change color. Most people agree that when PH ≥ 9.0, the protein would be false positive. To determine if urinary dipsticks accurately assess protein concentrations, especially in alkaline urine, we used the sulfosalicylic acid method to evaluate it. Our data showed that false-positive results were significantly higher in urine specimens when PH ≧ 8.0. Jason L. Robinson etc. indicated that urine protein results were not affected by NaOH alkalization up to pH 10.9, and false-positive protein occurred at pH 9.9 (Robinson et al., 2019). They were different from our results, maybe there were no additional alkaline substances in our study, and the protein module resistant to interference by PH was not good enough.

All of these differences remind us that to provide more accuracy of the results and reduce misunderstanding; we should pay more attention to our experiment conditions, and give some comments which set in our reports:

1. When the concentration of urine vitamin C was 5.7 mmol/L, it would interfere with the chemical result of GLU, BIL, and BLD.
2. When DIRUI dipsticks showed the concentration of vitamin C 2.8 mmol/L, it would interfere with the chemical result of GLU and BLD.
3. When DIRUI dipsticks showed the concentration of vitamin C, either 1.4 mmol/L or 0.6 mmol/L, it would only interfere with the chemical result of BLD.
4. Urine PH ≧ 8.0, the false-positive protein was confirmed by the sulfosalicylic acid method.
5. Urine protein is positive with the sulfosalicylic acid method; we suggested that patients should do a 24-h urine collection of protein test.

4 Conclusions

Mix iodine-potassium iodide solution and vitamin C, it would have a red-ox reaction, and adequate iodine-potassium iodide solution doesn't cause false-positive results in urine dry chemistry tests. Some experiments succeeded in our laboratory, but it was difficult to confirm the iodine-potassium iodide solution's detailed concentration.

To minimize the risk of vitamin C and PH, we should do more to help guarantee the accuracy of the urinalysis results and communicate with doctors more to achieve effective communication.

Ethical approval

The study was approved by the Ethical Committee of our school.

Conflicts of interest

The authors declare that they have no conflicts of interests.

Author contributions

DF designed the study and wrote 1st draft; JL and JC collected data and did the methodology. XC did the data analysis and prepared the instruments. HY gave comments on the study conception, did critical reviews and rewriting on the manuscript. All the authors agreed on the submission of the manuscript.

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