



## RESEARCH ARTICLE

# Ascorbic acid—A black hole of urine chemistry screening

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**Background:** Study was performed in order: (i) to assess the comparability of glucose, bilirubin, hemoglobin, leukocyte esterase, and protein; (ii) to assess accuracy of glucose, bilirubin, hemoglobin, leukocyte esterase, and protein; and (iii) to evaluate interference of ascorbic acid on the glucose, bilirubin, hemoglobin, and nitrite determination using 2 different dipsticks: iChem Velocity, Iris Diagnostics and Combur-10M, Roche Diagnostics.

**Methods:** Random urine specimens were included in the study. Comparability, accuracy, and ascorbic acid interference testing were performed.

**Results:** Obtained results have shown almost perfect agreement for all parameters between 2 dipsticks in samples with negative ascorbic acid. Agreement in samples with positive ascorbic acid was not acceptable for bilirubin, protein, nitrite, and hemoglobin. Accuracy was not acceptable for hemoglobin and leukocyte esterase on both dipsticks. Ascorbic acid interference examination has shown that intensity of interference differs between dipsticks. Ascorbic acid interferes with glucose, hemoglobin, nitrite, and bilirubin at different concentrations causing false-negative results.

**Conclusion:** Obtained results indicate that it is necessary to determine diagnostic accuracy of used dipstick in order to define purpose of urinalysis. It is very important to choose dipstick with ascorbic acid indicator and to examine ascorbic acid impact on dipstick analytes independently of manufacturer claims.

**KEYWORDS**

ascorbic acid, diagnostic accuracy, interference, patient safety, urinalysis

## 1 | INTRODUCTION

Urinalysis was the first laboratory test performed in medicine and still remains the most performed medical test used as a screening tool in diagnosis of urinary tract infections, kidney disorders, liver problems, diabetes, or other metabolic conditions.<sup>1</sup> The reasons for this are as follows: noninvasive sampling, low cost, simplicity of performance, and short turnaround time.

Complete urinalysis includes physical (color and clarity), chemical (specific gravity, identification of hemoglobin, glucose, pH, bilirubin, urobilinogen, ketones, nitrites, leukocyte esterase, and protein), and microscopic urine examination (detection of cells, crystals, casts, bacteria, and yeasts).

Automatization of chemical examination of urinalysis in the past few decades has led to its better sensitivity and specificity, and recent automatization of microscopic examination has further improved diagnostic accuracy of the urinalysis.<sup>2,3</sup>

Like all screening tests, urinalysis claims to have a high negative predictive value. Recent risk analysis study by Miler et al<sup>4</sup> has shown that microscopic urine examination can be excluded if urine has negative dipstick test results for protein, leukocyte esterase, nitrite, and hemoglobin. Other studies also confirm urinalysis as reliable screening method for diagnosis of urinary tract infections and diabetes mellitus.<sup>5,6</sup>

Despite the above, it is important to keep in mind that results of urinalysis obtained by dipsticks are semiquantitative with limited

analytical sensitivity and still there is a large heterogeneity between dipsticks available on the market.

Moreover, it is known that there is some interference which can impact performance of chemical examination. The most common is ascorbic acid interference on dipstick performance for detection of glucose, hemoglobin, nitrite, and bilirubin.<sup>7,8</sup>

Therefore, we performed our study in order: (i) to assess the comparability of glucose, bilirubin, hemoglobin, leukocyte esterase, and protein; (ii) to assess accuracy of glucose, bilirubin, hemoglobin, leukocyte esterase, and protein; and (iii) to evaluate interference of ascorbic acid on the glucose, bilirubin, hemoglobin, and nitrite determination using 2 different dipsticks: iChem Velocity, Iris Diagnostics and Combur-10M, Roche Diagnostics.

## 2 | MATERIALS AND METHODS

### 2.1 | Materials

Random urine specimens, with requested urinalysis, sent to the University Department of Clinical Chemistry in Medical School University Hospital Sestre Milosrdnice, Zagreb, Croatia, during September and October 2016, were included in the study.

Urine samples were delivered to the laboratory in urine cups or tubes (Greiner Bio-One GmbH, Kremsmunster, Austria). Combur-10M dipsticks (Roche Diagnostics, GmbH, Mannheim, Germany) and iChem Velocity dipsticks (Iris Diagnostics, Chatsworth, USA) were used for chemical examination of urine. Urine analyzers Cobas u411 (Roche Diagnostics, GmbH) and Iris IQ200 (Iris Diagnostics) were used for dipstick analyzing. Analytical sensitivities and reported values for each dipstick are presented in Table 1. Tests were performed as described in manufacturer's instruction including recommended procedures for quality control and maintenance.

### 2.2 | Comparison testing

Comparability between dipsticks was evaluated on 406 patient's samples for bilirubin, hemoglobin, glucose, protein, leukocyte esterase, and nitrite. All urine samples with requested urinalysis and minimal volume of 15 mL (sufficient volume to ensure complete urinalysis on both analyzers including microscopic exam) were included in the study. Samples were recruited in the study until there were at least 10 samples per each report category for each tested analyte. Reported category was defined according to the manufacturer instruction (normal/negative, 1+, 2+, 3+). Combur-10M dipstick was used as a reference method.

### 2.3 | Accuracy testing

Accuracy was evaluated using 229 patient's samples for bilirubin and hemoglobin, 213 for glucose, 43 samples for protein, and 40 urine samples for leukocyte esterase. Only samples with minimal volume of 15 mL (sufficient volume to ensure complete urinalysis on both analyzers including microscopic exam) were included in the study.

Accuracy was tested for both dipsticks by comparing the results of patient's samples obtained by dipstick and using reference methods on Architect c8000 (Abbott, IL, USA) and XN-1000 (Sysmex, Kobe, Japan). Reference methods used were as follows: hexokinase for glucose, diazonium salt for total bilirubin, benzethonium chloride for urine protein, and sample interference indices saline protocol for hemoglobin (original Abbott reagents). Leukocytes were determined using flow cytometry in body fluids mode on XN-1000. Urine samples with positive ascorbic acid were excluded from the accuracy testing.

### 2.4 | Ascorbic acid interference

Ascorbic acid interference was tested for bilirubin, hemoglobin, glucose, and nitrite for both tested dipsticks. For ascorbic acid interference examination, stock solutions of each tested analyte were prepared. The materials used were as follows: bilirubin (Sigma/Aldrich, MO, USA), hemoglobin (whole blood), anhydrous glucose (Kemig d.o.o, Croatia), sodium nitrite (Kemika d.o.o, Croatia), and Vitamin C500 (Worwag pharma GmbH&Co.KG, Böblingen, Germany). Specific analyte concentration (bilirubin, hemoglobin, glucose, and nitrite) of urine samples were prepared by adding appropriate volume of stock solution to the negative urine pool sample. Negative urine pool sample was prepared from negative urine samples which were negative/normal for all tested analytes on each tested dipstick. Prepared urine samples with desirable analyte concentrations (glucose: 50, 100, and 300 mg/dL; hemoglobin: 0.03, 0.06, and 0.10 mg/dL; nitrite: 0.10 and 0.20 mg/dL; and bilirubin: 1.8 and 4.0 mg/dL) were then spiked with ascorbic acid (20, 40, 50, 100, 200, or 500 mg/dL). Each urine sample with specific combination of analyte/ascorbic acid concentration was tested in duplicate. Concentrations of each analyte (except nitrite and ascorbic acid) in prepared urine samples were tested on Architect c8000. Nitrite and ascorbic acid concentrations were confirmed by dipstick determination.

### 2.5 | Statistical analysis

For evaluation of comparability and accuracy, Kappa coefficients with 95% confidence intervals were calculated. Lower limit of 95% confidence interval of Kappa  $\geq 0.6$  (moderate level of agreement) was considered acceptable.<sup>9</sup>

For comparability testing, results were divided into 2 subgroups according to the presence of ascorbic acid and evaluated separately.

For accuracy testing, results of analyte determination on reference analyzers were converted to report value categories according to Table 1.

Additionally, to evaluate accuracy, diagnostic specificities and sensitivities were calculated according to the following formulas:

1. Diagnostic sensitivity =  $100 \times ((\text{true positive}/(\text{true positive} + \text{false negative})))$
2. Diagnostic specificity =  $100 \times ((\text{true negative}/(\text{true negative} + \text{false positive})))$

**TABLE 1** Analytical sensitivities and reported values for each analyte on Combur-10M and iChem Velocity dipsticks

Analyte	iChem Velocity			Combur Test 10M		
	Analytical sensitivity	Reported concentrations	Reported categories	Analytical sensitivity	Reported concentrations	Reported categories
Bilirubin	0.5 mg/dL	<1.0	Negative	0.4-0.6 mg/dL	<1	Negative
		1-3	1+		1-3	1+
		3-6	2+		3-6	2+
		>6	3+		>6	3+
Urobilinogen	2 mg/dL	<2	Normal	1-1.6 mg/dL	<1	Normal
		2-4	1+		1-4	1+
		4-8	2+		4-8	2+
		>8	3+		>8	3+
Ketones	5 mg/dL	<5	Negative	3-6 mg/dL	<5	Negative
		5-20	1+		5-15	1+
		20-60	2+		15-50	2+
		>60	3+		>50	3+
Glucose	20 mg/dL	<50	Normal	30-40 mg/dL	<50	Normal
		50-100	1+		50-100	1+
		100-1000	2+		100-1000	2+
		>1000	3+		>1000	3+
Protein	10 mg/dL	<50	Negative	12-18 mg/dL	<25	Negative
		50-100	1+		25-75	1+
		100-300	2+		75-150	2+
		>300	3+		>150	3+
Hemoglobin	0.015 mg/dL	<0.01	Negative	0.012-0.030 mg/dL	<0.01	Negative
		0.01-0.03	1+		0.01-0.03	1+
		0.03-0.06	2+		0.03-0.06	2+
		>0.06	3+		>0.06	3+
Nitrite	0.06 mg/dL	<0.1	Negative	0.05-0.07 mg/dL	<0.1	Negative
		>0.1	Positive		>0.1	Positive
Leukocyte esterase	25 WBC/ $\mu$ L	<25	Negative	20-25 WBC/ $\mu$ L	<25	Negative
		25-75	1+		25-100	1+
		75-500	2+		100-500	2+
		>500	3+		>500	3+

WBC, white blood cells.

**TABLE 2** Comparability of tested analytes on Combur-10M and iChem Velocity dipsticks depending on ascorbic acid (AA) presence in urine sample

Analyte	AA negative N = 370	AA positive N = 36
Bilirubin	0.778 (0.701-0.855)	0.442 (-0.009 to 0.893)
Hemoglobin	0.758 (0.708-0.808)	0.646 (0.266 to 1.000)
Glucose	0.915 (0.884-0.947)	0.890 (0.798 to 0.982)
Protein	0.738 (0.687-0.788)	0.516 (0.264 to 0.767)
Leukocyte	0.874 (0.842-0.905)	0.873 (0.780 to 0.965)
Nitrite	0.753 (0.644-0.862)	0 (0 to 0)

Data are presented as weighted kappa and 95% confidence interval. AA, ascorbic acid.

3. Diagnostic accuracy =  $100 \times ((\text{true positive} + \text{true negative}) / (\text{true positive} + \text{false positive} + \text{true negative} + \text{false negative}))$

If the reported category (positive or negative) of the results obtained by the dipstick and by reference method was the same, it is considered as true positive or true negative, and if result was not in the same reported category on tested strip in comparison with the reference method determination, it was considered as false positive or false negative.

Interference was claimed at the specific combination of analyte/ascorbic acid concentration where results obtained by dipstick were not in concordance with the results obtained in the urine sample with the same analyte concentration but without ascorbic acid.

MedCalc (v12.7.2.0, Ostend, Belgium) was used for statistical analysis.

### 3 | RESULTS

Obtained results have shown substantial to almost perfect agreement for all tested parameters between 2 dipsticks in urine samples with negative ascorbic acid (Table 2). On the other hand, agreement between tested dipsticks in urine samples with positive ascorbic acid was not acceptable for bilirubin, protein, nitrite, and hemoglobin. Furthermore, even though agreement was acceptable for glucose and leukocyte esterase, Kappa coefficients were lower than in urine samples with negative ascorbic acid (Table 2).

Results of accuracy testing are presented in Table 3.

Based on the results of Kappa values, accuracy was not acceptable for hemoglobin and leukocyte esterase on both dipsticks. Diagnostic sensitivity was the lowest for hemoglobin on iChem Velocity (68.2%). All other parameters have shown diagnostic sensitivity from 72% to 100%. Diagnostic specificities were the lowest for leukocyte esterase and hemoglobin on both dipsticks, and the specificities for other tested parameters ranged from 75% to 100% (Table 4).

Results of ascorbic acid interference examination have shown that intensity of interference differs between dipsticks. Ascorbic acid interferes with glucose, hemoglobin, nitrite, and bilirubin at different concentrations causing false-negative results (Table 5).

**TABLE 3** Results of accuracy testing

Analyte	N	iChem Velocity			Architect			Combur-10M			Architect						
		Negative/normal	1+	2+	3+	Negative/normal	1+	2+	3+	Negative/normal	1+	2+	3+				
Glucose	213	180	12	16	5	183	8	17	5	183	10	11	9	182	11	16	4
Bilirubin	229	190	28	7	4	177	44	4	4	177	31	15	6	177	44	4	4
Protein	43	8	24	11	0	6	26	8	3	10	12	13	8	15	10	12	6
Leukocyte esterase	40	2	11	21	6	7	11	15	7	0	12	15	13	7	15	11	7
Hemoglobin	229	108	/	/	121	126	/	/	103	88	/	/	141	126	/	/	103

**TABLE 4** Diagnostic accuracy of tested analytes on Combur-10M and iChem Velocity dipsticks

Analyte	iChem Velocity					Combur-10M				
	N	Weighted kappa (95% CI)	Specificity (%)	Sensitivity (%)	Accuracy (%)	Weighted kappa (95% CI)	Specificity (%)	Sensitivity (%)	Accuracy (%)	
Glucose	213	0.943 (0.913 to 0.973)	98.4	100	95	0.926 (0.880 to 0.972)	100	99.5	99	
Bilirubin	229	0.785 (0.674 to 0.895)	96.2	72	88	0.805 (0.726 to 0.883)	93.2	80	89	
Protein	40	0.795 (0.685 to 0.905)	100	95	95	0.898 (0.835 to 0.961)	75	100	88	
Leukocyte esterase	40	0.603 (0.405 to 0.800)	58.3	100	88	0.409 (0.206 to 0.612)	50	100	83	
Hemoglobin	229	0.010 (-0.118 to 0.138)	65.6	68.2	50	0.044 (-0.078 to 0.166)	62.7	73.6	51	

iChem Velocity dipsticks were the most impacted by ascorbic acid interference when hemoglobin, bilirubin, and nitrite were tested, and Combur-10M dipsticks were more impacted when glucose, hemoglobin, and bilirubin were tested.

Results of our study have shown that ascorbic acid interferes with glucose measured on Combur-10M at 20 mg/dL and above 200 mg/dL on iChem Velocity, with hemoglobin at 20 mg/dL on both tested dipsticks, with nitrite at 50 mg/dL on iChem Velocity and above 200 mg/dL on Combur-10 M, and with bilirubin at 50 mg/dL on both tested dipsticks (Table 5).

## 4 | DISCUSSION

The main finding of our study was that tested dipsticks can be used interchangeably despite differences in specifications only if samples are negative for ascorbic acid. Accuracy of tested dipsticks is not satisfactory for their usage as screening method for some analytes and interference study showed that ascorbic acid has different impact on dipsticks and impact is not in concordance with manufacturer claims.

Findings above indicate that it is important to be careful when purpose of urine chemistry testing is considered. Although urinalysis is used as a screening in diagnosis of urinary tract infections, kidney disorders, liver problems, diabetes, or other metabolic conditions, our study indicates that there are several problems which we should keep in mind when results of dipstick tests are interpreted.

First of all, definition of screening test means that test can be used in a general population who are not aware of disease to identify the disease.<sup>10</sup> In the other words, diagnostic sensitivity of these tests should be high. Our results have shown low diagnostic sensitivity for hemoglobin and bilirubin for both tested dipsticks. This means that these tested strips should not be used as a screening for detection of hematuria and hyperbilirubinemia. Even though other tested parameters have shown high diagnostic sensitivity, leukocyte esterase and hemoglobin on both tested dipsticks have shown low specificity which means that positive leukocyte esterase and hemoglobin should be confirmed by confirmation method (eg, urine microscopy).<sup>11</sup>

In the study of Zamanzad, urine samples were screened for protein, glucose, hemoglobin, and nitrite using standard dipsticks (Uriyab-8, Bakhtar Chemistry Co., Kermanshah, Iran). Author concluded that dipstick urinalysis can be a reliable screening method for diagnosis of urinary tract infections and diabetes mellitus but not for proteinuria as a marker of renal insufficiency or renal target organ damage.<sup>5</sup> Our results also confirmed that urinalysis is reliable screening method for diabetes mellitus. Screening of urinary tract infections using leukocyte esterase results is suitable according to our results, but according to the low diagnostic specificity, results should be confirmed. Diagnostic accuracy of proteinuria screening depends on used dipstick. Rehmani in his study evaluated 984 samples of urine to determine sensitivity and specificity of tested dipsticks for leukocyte esterase and nitrite in comparison with urine culture. Author concluded that dipstick alone cannot accurately predict urinary tract infection in emergency department.<sup>12</sup>

**TABLE 5** Ascorbic acid interference on tested analytes on iChem Velocity and Combur-10M dipsticks

Analyte	Analyte concentration (mg/dL)		Ascorbic acid concentration	iChem Velocity	Combur-10M	
	iChem Velocity reported category <sup>a</sup>	Combur-10M reported category <sup>b</sup>				
Glucose	50	1+ <sup>a</sup> 1+ <sup>b</sup>	0	Normal	1+	
			20	Normal	Normal	
			50	Normal	Normal	
			100	Normal	Normal	
			200	Normal	Normal	
			500	Normal	Normal	
			100	2+	2+	
	2+ <sup>a</sup> 2+ <sup>b</sup>	2+	0	1+	2+	
			20	1+	1+	
			50	1+	1+	
			100	1+	Normal	
			200	1+	Normal	
			500	1+	Normal	
			300	2+	3+	
	3+ <sup>a</sup> 3+ <sup>b</sup>	3+	0	2+	3+	
			20	2+	2+	
			50	2+	3+	
			100	2+	2+	
			200	2+	2+	
			500	Normal	2+	
			0.03	2+	2+	
	Hemoglobin	2+ <sup>a</sup> 2+ <sup>b</sup>	2+	0	2+	2+
				20	1+	Negative
				40	1+	Negative
200				Negative	Negative	
500				Negative	Negative	
0.06				2+	2+	
3+ <sup>a</sup> 3+ <sup>b</sup>		3+	0	2+	2+	
			20	1+	2+	
			40	1+	2+	
			200	Negative	1+	
			500	Negative	Negative	
			0.10	2+	3+	
3+ <sup>a</sup> 3+ <sup>b</sup>	3+	0	2+	3+		
		20	2+	3+		
		40	1+	3+		
		200	Negative	3+		
		500	Negative	Negative		
		0.10	2+	3+		
Nitrite	0.10 Negative <sup>a</sup> Negative <sup>b</sup>	Negative	0	Positive	Negative	
			20	Negative	Negative	
			40	Negative	Negative	
			200	Negative	Negative	
			500	Negative	Negative	
			0.20	Positive	Positive	
	Positive <sup>a</sup> Positive <sup>b</sup>	Positive	0	Positive	Positive	
			20	Positive	Positive	
			40	Negative	Positive	
			200	Negative	Positive	
			500	Negative	Negative	
			0.20	Positive	Positive	

(Continues)

**TABLE 5** (Continued)

Analyte	Analyte concentration (mg/dL) iChem Velocity reported category <sup>a</sup> Combur-10M reported category <sup>b</sup>	Ascorbic acid concentration	iChem Velocity	Combur-10M
Bilirubin	1.8	0	2+	3+
	1 <sup>+</sup> <sup>a</sup>	20	1+	2+
	1 <sup>+</sup> <sup>b</sup>	40	Negative	1+
		200	Negative	1+
		500	Negative	Negative
	4.0	0	2+	3+
	2 <sup>+</sup> <sup>a</sup>	20	2+	3+
	2 <sup>+</sup> <sup>b</sup>	40	1+	2+
		200	1+	2+
		500	Negative	1+

Highlighted fields present interference points stated by the manufacturers.

On contrary, Devillé et al<sup>6</sup> concluded that the urine dipstick alone seems to be useful in all populations to exclude the presence of infection if the results for both nitrites and leukocyte esterase are negative

As mentioned above, our results have shown that the diagnostic specificity of tested dipsticks for leukocyte esterase is low and not suitable for diagnosis of urinary tract infections. We did not test diagnostic accuracy of nitrite, but it is possible that combination of these 2 parameters improves diagnostic accuracy of urinalysis for urinary tract infections.

All these results indicate that dipsticks are not suitable for screening of all chemical urinalysis parameters and that all dipsticks do not have same specifications. Furthermore, to reduce costs, "negative" urinalysis test results are not usually followed up with additional confirmatory tests.<sup>4,13,14</sup>

Although iChem Velocity dipsticks claim to be more sensitive for protein, glucose, and hemoglobin, obtained diagnostic sensitivities for those parameters are the same, or even slightly worse than on Combur-10M dipsticks.

All facts stated above indicate that analytical sensitivity claimed by manufacturer does not provide information about diagnostic accuracy of urine dipsticks.

Our results have also shown that ascorbic acid interference has different impact on different dipsticks and that real interference impact is seriously overlooked in the manufacturer claims.

Roche states that ascorbic interference is almost completely eliminated for hemoglobin and glucose and that only very high concentration could interfere with bilirubin determination on Combur-10M dipsticks. iChem Velocity insert states that ascorbic acid concentrations above 20 mg/dL can cause strong interference with glucose and hemoglobin on iChem Velocity dipsticks.

Although Roche states that there is no interference of ascorbic acid for hemoglobin and glucose, our results demonstrate that even low dose of ascorbic acid has impact on these analytes on Combur-10M dipsticks. For bilirubin where Roche quotes that only high concentrations of ascorbic acid interfere with its determination, our study also demonstrates interfering impact of even low doses of ascorbic acid. iChem Velocity states strong interference for hemoglobin and glucose,

and results of our study confirmed this claim. Ascorbic acid interferes with hemoglobin even at low concentrations; however, high concentrations of ascorbic acid are required to cause interference of glucose on iChem Velocity dipsticks. Manufacturer does not quote interference on nitrite and bilirubin, but results of our study indicate that these 2 parameters are even more impacted by ascorbic acid than glucose.

Furthermore, iChem Velocity dipsticks are most impacted by ascorbic acid when hemoglobin, bilirubin, and nitrite are tested, and Combur-10M dipsticks are more impacted by ascorbic acid when glucose, hemoglobin, and bilirubin are tested.

Despite manufacturer claims, our results have shown that Combur-10M strips are more sensitive on ascorbic acid interference for glucose, hemoglobin, and bilirubin than iChem Velocity dipsticks.

Ascorbic acid (vitamin C) is frequently found in clinical urine samples and can interfere with the dipstick results.<sup>15</sup> Previous studies have demonstrated that even modest ingestion of vitamin C supplements (350-1000 mg/d) can cause high concentration of vitamin C in urine which interferes with dipstick determination of glucose and hemoglobin. Moreover, all subjects who ingested a dose of 350 to 1000 mg of vitamin C per day had concentrations of 25 mg/dL or more of vitamin C in urine.<sup>16,17</sup> Most of the studies which investigated ascorbic acid impact on urinalysis were performed 10-20 years ago,<sup>7,15-17</sup> which makes our investigation even more important.

Berg demonstrated that with 30 mg/dL vitamin C, 23 of 30 specimens with glucose levels of 50 mg/dL showed false-negative results on Roche chemistry strips.<sup>18</sup>

Brigden et al<sup>19</sup> also reported false-negative results for glucose and hemoglobin on 4 different urine dipsticks. One of the tested strips was Chemstrip (US brand of the Combur-Test by Roche Diagnostics), and interference effect of ascorbic acid was similar to effect of ascorbic acid on glucose and hemoglobin in our study.

In the study of Nagel et al,<sup>20</sup> 5 different dipsticks were investigated for ascorbic acid interference on glucose and hemoglobin. Combur-10M test strips have shown same interference effect on glucose and hemoglobin as well as in our study.

Ko et al<sup>8</sup> have also shown that overlooking the presence of vitamin C in urine may lead to potentially serious false-negative results, especially for glucose and hemoglobin.

Milan et al examined impact of ascorbic acid on the performance of 4 different dipsticks. Results of their study also confirmed that ascorbic acid can cause false-negative results for glucose, hemoglobin, nitrite, and bilirubin, and those different dipsticks demonstrate different negative effect of ascorbic acid on certain analytes which is not in consistent with manufacturer claims.<sup>7</sup> However, the results of this study have shown similar impact of ascorbic acid on iChem Velocity dipstick for glucose and hemoglobin, but they did not find impact on nitrite and bilirubin while results of our study have shown that even low concentration of ascorbic acid in urine interferes with nitrite and bilirubin dipstick tests.

Together with the results of our study, above-mentioned results lead to the conclusion that ascorbic acid is very important interfering factor that can cause false-negative results for urine chemistry analysis. That is why the possibility of the ascorbic acid detection in urine offered by iChem Velocity dipsticks could be of great help to avoid misdiagnosis. For urine specimens positive for ascorbic acid, the results of chemistry urinalysis should be reported with a comment in order to help physicians to interpret results appropriately. The other solution is a use of ascorbic acid-resistant dipsticks, but their shortcoming is that they do not eliminate possible interference of high ascorbic acid concentrations.<sup>13</sup> It is also important to emphasize that ascorbic acid interference on used dipsticks has to be confirmed in laboratory despite manufacturer claims.

There are also a few limitations of the study. First of all, it was not possible to check is there any other interfering factor besides ascorbic acid in tested urine samples. Furthermore, ascorbic acid interference was not tested in urine samples of patients who ingested vitamin C, but the results of comparability testing have shown that ascorbic acid has effect on tested dipsticks in clinical urine samples.

## 5 | CONCLUSION

Results of our study indicate that it is necessary to determine diagnostic accuracy of used urine dipsticks in laboratory and in concordance with obtained results define purpose of urinalysis. It is also of extraordinary importance to choose dipstick with ascorbic acid indicator and also to examine ascorbic acid impact on dipstick analytes independently of manufacturer claims.

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