P 20387 E

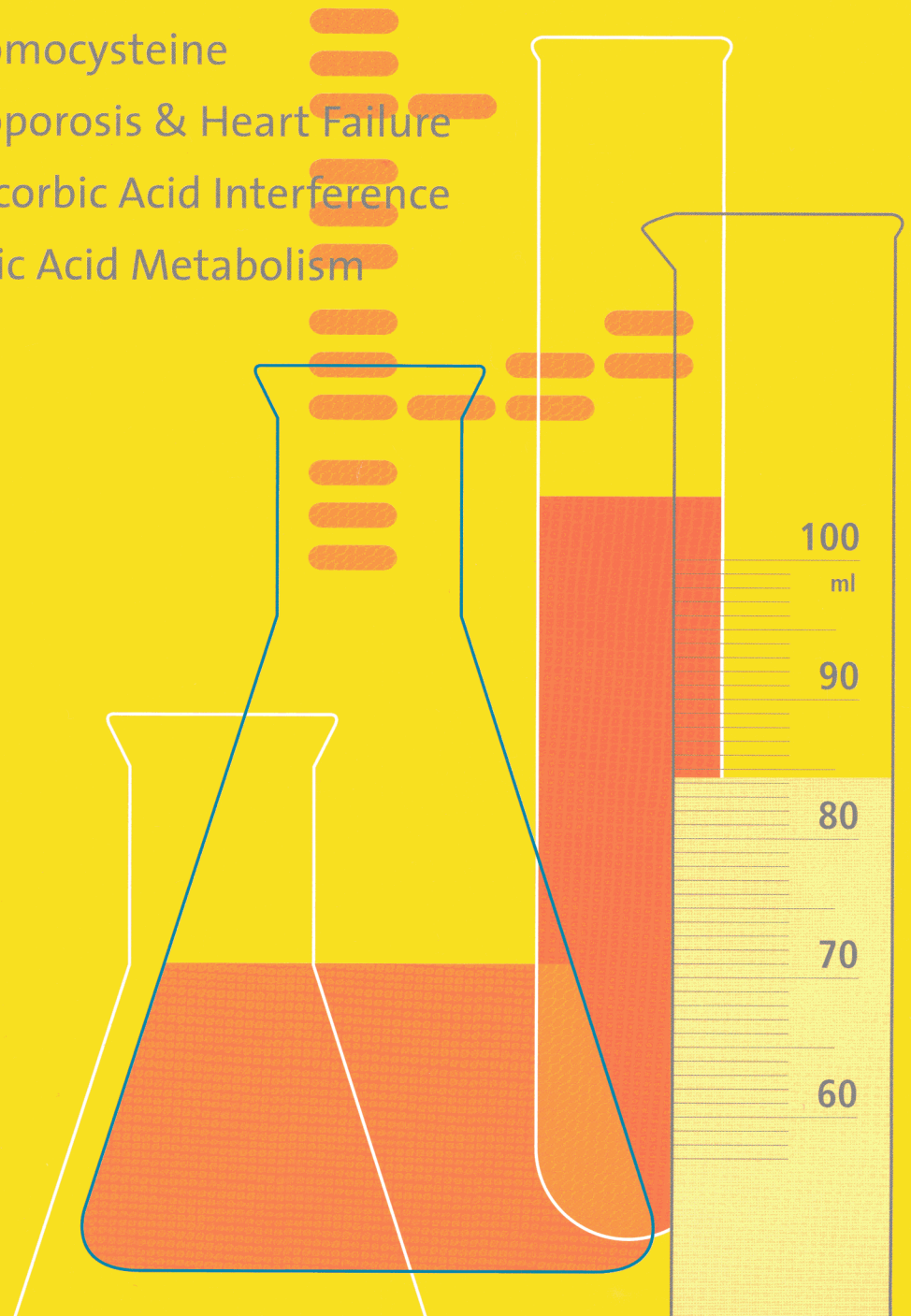
3+4/2006

Clinical Laboratory

www.clin-lab-publications.de

VOLUME 52

- Anemia, Thalassemia
- Fibrinolysis in Newborns and Children
- Acute Myeloid Leukemia
- Bone Turnover & Homocysteine
- Homocystein, Osteoporosis & Heart Failure
- Urine Test Strips, Ascorbic Acid Interference
- Inborn Errors of Sialic Acid Metabolism
- Journal Club



ISSN 1433-6510

Clin. Lab. 2006;52:149-153

ORIGINAL ARTICLE

Investigations of Ascorbic Acid Interference in Urine Test Strips

DIETMAR NAGEL[1], DIETER SEILER[1], EWALD F. HOHENBERGER[2], MANFRED ZIEGLER[2]

[1] *Klinikum Ludwigshafen, Institut für Klinische Chemie, Ludwigshafen, Germany*
[2] *Roche Diagnostics GmbH, Mannheim, Germany*

SUMMARY

Ascorbic acid at higher concentration in urine samples can lead to false negative results in a number of urine tests, with a potential risk of clinical findings being overlooked, particularly with glucose and hemoglobin. For this reason, the ascorbic acid status of urine samples should always be routinely known so as to establish what adjustment needs to be made. A much better approach, however, is to use a test which is by design largely resistant to ascorbic acid. We compared five very common 10-parameter urine test strips from different manufacturers. The results of this study show that of the strips tested, only the product Combur-Test from Roche Diagnostics is largely resistant to ascorbic acid interference. Even lowest – but clinically relevant – concentrations of erythrocytes (10/μL), hemoglobin (0.03 mg/dL), and glucose (50 mg/dL) were correctly detected with concentrations of up to 400 mg/L ascorbic acid. Higher analyte concentrations correctly reacted positive even in the presence of up to 1000 mg/L ascorbic acid. (Clin. Lab. 2006;52:149-153)

KEY WORDS

Urine test strips, ascorbic acid, interference, glucose, hemoglobin, erythrocytes

INTRODUCTION

The ingestion of high doses of ascorbic acid – as a common constituent of food, or in the form of tablets and fruit juices – is widespread today. As a result, high ascorbic acid concentrations of ≥ 400 mg/L are increasingly being found in the urine samples tested by laboratories. Urine test strips for visual estimation of urine analytes are still widely used in the wards of many hospitals and by physicians in their practice.
Ascorbic acid on its part is known to be able to interfere strongly in redox processes of certain dye indicator reactions of urine test strips, particularly in glucose and hemoglobin indications as documented in in-vitro experiments (1). Most investigations for interference of ascorbic acid with urinary parameters like hemoglobin and glucose are almost 20 years old (2, 3).

Manuscript accepted September 9, 2005

The aim of this study was to evaluate which nowadays available tests of top sellers in the urinalysis market are by design largely resistant to ascorbic acid.

MATERIAL AND METHODS

The study was performed in the Institute of Clinical Chemistry of the Klinikum Ludwigshafen.
Five very common 10-parameter urine test strips from different manufacturers were compared by visual reading of erythrocytes, hemoglobin and glucose results at different sample analyte concentrations at varying urine ascorbic acid concentrations.

Reagent carriers

The following test strips were used for visual reading (abbreviations in brackets)

Arkray, Inc	Aution 10 EA	(AA)
Bayer Diagnostics	Multistix 10SG	(MX)
Macherey & Nagel	Medi-Test Combi 10SGL	(MN)
Roche Diagnostics	Combur[10] Test UX	(UX)
YD Diagnostics	Uriscan 10SGL	(YD)

Table 1: Prepared concentration ranges tested

Ascorbic acid	0	100	200	400	1000	mg/L
Glucose	50	100	300			mg/dL
Hemoglobin	0.03	0.075	0.15	0.30		mg/dL
(corresponding)	(10)	(25)	(50)	(100)		(erythrocytes/μL)
Erythrocytes	10	25	50	250		(erythrocytes/μL)

Table 2: Concentration scheme according to the manufacturers

	Bayer: Multistix 10 SG						
Glu	NEG	100	250	500	1000	> 2000	mg/dL
		Trace	**+**	**2+**	**3+**	**4+**	
Ery	NEG	10		80			Ery/μL
		Trace+		**2+**			
Hb	NEG	10	25	80	200		Ery/μL
		0.03	0.075	0.24	0.6		mg/dL
		Trace	**+**	**2+**	**3+**		

	Macherey & Nagel: Combi 10 SGL						
	NEG	NORM.	50	150	500	>1000	mg/dL
	NEG	ca.5-10	ca. 50	ca. 250			Ery/μL
		+	**2+**	**3+**			
	NEG	ca. 10	ca. 50	ca. 250			Ery/μL
		ca.0.03	ca.0.15	ca.0.75			mg/dL
		+	**2+**	**3+**			

	Arkray: Aution 10 EA						
Glu	NORM.	50	100	200	500	1000	mg/dL
		+/-	**+**	**2+**	**3+**	**4+**	
Ery	NEG	**+**	**2+**	**3+**			
Hb	NEG	20	66	330			Ery/μL
		0.06	0.2	1.0			mg/dL
		+	**2+**	**3+**			

	Roche: Combur[10]Test UX					
	NORM.	50	100	300	1000	mg/dL
		+	**2+**	**3+**	**4+**	
	NEG	ca. 5-10	ca.25	ca. 50	ca. 250	Ery/μL
		+	**2+**	**3+**	**4+**	
	NEG	ca.10	ca.25	ca. 50	ca. 250	Ery/μL
		0.03	0.075	0.15	0.75	mg/dL
		+	**2+**	**3+**	**4+**	

	YD: Uriscan 10 SGL						
Glu	NEG	100	250	500	1000	>2000	mg/dL
		+/-	**+**	**2+**	**3+**	**4+**	
Ery	NEG		**+**	**2+**	**3+**		
Hb	NEG		10	50	250		Ery/μL
			0.03	0.15	0.75		mg/dL
			+	**2+**	**3+**		

All test strips were used according to the manufacturers' specifications, especially by regarding individual test reading times.

Sample material

The sample material used was pooled ascorbic acid-free native urine. For the experimental design, stock solutions of glucose (2000 mg/dL), erythrocytes (10000 erythrocytes/μL) lysed erythrocytes (10 mg/dL Hb) and ascorbic acid (2000 mg/L) were prepared by adding the amount of the corresponding substances to pooled urine to establish the concentration ranges shown in Table 1. Hemoglobin solution was prepared from diluted whole blood by supersonic hemolytic treatment. Erythrocyte solution was prepared by washing the erythrocytes three times with saline solution. The exact erythrocyte count was determined by a Sysmex 2100 instrument.

Each set of urine concentrations was portioned into 15 mL Falcon tubes according to the number of examined test strips.

Table 3: Visual readings of different glucose and ascorbic acid concentrations

Test	Glucose 50 mg/dL					Glucose 100 mg/dL					Glucose 300 mg/dL				
	Ascorbic Acid (mg/L)					Ascorbic Acid (mg/L)					Ascorbic Acid (mg/L)				
	0	100	200	400	1000	0	100	200	400	1000	0	100	200	400	1000
UX	100	50	50	50	0	100	100	100	50	50	300	300	300	300	300
MX	0	0	0	0	0	100	100	100	0	0	500	250	250	250	100
MN	50	50	0	0	0	50	50	50	0	0	500	150	150	150	0
AA	0	0	0	0	0	50	50	50	0	0	100	200	100	100	50
YD	100	0	0	0	0	100	250	100	0	0	500	500	500	500	250

Table 4: Visual readings of different erythrocytes and ascorbic acid concentrations

Test	Int. Erythrocytes 10Ery/µL					Int. Erythrocytes 25Ery/µL					Int. Erythrocytes 50Ery/µL				
	Ascorbic Acid (mg/L)					Ascorbic Acid (mg/L)					Ascorbic Acid (mg/L)				
	0	100	200	400	1000	0	100	200	040	1000	0	100	200	400	1000
UX	+	+	+	+	+	2+	+	+	+	+	2+	3+	3+	2+	2+
MX	+	+	+	+	+	2+	2+	+	+	+	2+	2+	>2+	2+	2+
MN	+	+	+	+	+	2+	+	+	+	+	2+	+	2+	+	+
AA	+	+	+	+	+	+	+	+	+	+	2+	2+	2+	2+	+
YD	0	(+)	0	0	0	+	+	(+)	0	0	+	+	+	+	+

Test	Int. Erythrocytes 250Ery/µL				
	Ascorbic Acid (mg/L)				
	0	100	200	400	1000
UX	4+	3+	4+	4+	4+
MX	>2+	>2+	>2+	>2+	>2+
MN	3+	3+	3+	3+	2+
AA	3+	3+	3+	3+	3+
YD	2+	2+	2+	2+	+

Reading mode

All color reactions were read independently by two operators who tested each set of urine specimens twice by using two identical strips of each manufacturer. In order to guarantee blinded trials, each set of urine specimens was randomized by a third party before. All determinations were performed within 4 hours.

All test strips were processed and read according to the manufacturers´ specifications.

Concentration ranges were determined by comparison of the respective reaction colors with the reference color blocks on the strip vial labels. If reaction colors were in between two reference blocks, the most adjacent color block had to be selected. Concentration values were recorded in randomized tables in form of concentration values or their corresponding arbitrary units specified individually by the manufacturers.

Evaluation

The concentration results recorded in the tables were "decoded" by linking with the respective randomizing scheme and rearranged according to the concentration scheme. The arbitrary units were translated into concentration values, if appropriate, according to specifications in order to ensure consistency (Table 2). The concentration results of both operators were in good agreement in the vast majority of cases.

RESULTS

Interference in Glucose Reactions

The results of visual readings at 50, 100 and 300 mg/dL glucose at stepwise addition of ascorbic acid 0 up to 1000 mg/L are summarized in Table 3.

Table 5: Visual readings of different hemoglobin and ascorbic acid concentrations

Test	Hemoglobin 0.03mg/dL=10Ery/µL					Hemoglobin 0.075mg/dL=25Ery/µL					Hemoglobin 0.15mg/dL=50Ery/µL				
	Ascorbic Acid (mg/L)					Ascorbic Acid (mg/L)					Ascorbic Acid (mg/L)				
	0	100	200	400	1000	0	100	200	400	1000	0	100	200	400	1000
UX	10	10	10	10	0	25	25	25	25	10	50	50	25	50	25
MX	10	10	0	0	0	25	10	10	0	0	80	80	25	25	25
MN	10	0	0	0	0	10	10	0	0	0	50	10	10	10	10
AA	0	0	0	0	0	20	20	20	0	0	330	66	66	66	20
YD	0	0	0	0	0	10	0	0	0	0	50	10	0	0	0

Test	Hemoglobin 0.3mg/dL=100Ery/µL				
	Ascorbic Acid (mg/L)				
	0	100	200	400	1000
UX	250	250	250	250	50
MX	200	200	80	80	25
MN	50	50	50	50	10
AA	330	330	330	330	66
YD	50	10	50	10	10

The results show that with no ascorbic acid present, only three out of the five tests were able by design to detect the pathologically relevant glucose concentration of 50 mg/dL. The Combur-Test urine test strips detected even that concentration range up to 400 mg/L ascorbic acid and consequently showed the highest resistance against ascorbic acid interference at higher glucose concentrations. The test results may differ at given concentrations due to individual gradation ranges of the tests (ref. to Table 2).

Interference in Erythrocyte Reactions

The results of visual readings at 10, 25, 50 and 250 erythrocytes/µL at stepwise addition of ascorbic acid 0 up to 1000 mg/L are summarized in Table 4.

A detailed analysis shows that the test strips of most manufacturers, except those of YD Diagnostics, detect erythrocytes correctly, even if it is sometimes difficult to assign the visual reading of erythrocyte spots to the correct concentration range, thus leading sometimes to a certain inconsistency for the different readings of erythrocytes.

Interference in Hemoglobin Reactions

The results of visual readings at 0.03, 0.075, 0.15 and 0.3 mg/dL hemoglobin at stepwise addition of ascorbic acid 0 up to 1000 mg/L are summarized in Table 5.

In the detailed analysis it is evident that with no ascorbic acid present, three out of the five tests are able by design to detect the pathologically relevant hemoglobin concentration of 0.03 mg/dL. The Combur-Test urine test strips detected even that concentration range up to 400 mg/L ascorbic acid and showed the best robustness against ascorbic acid interference at higher hemoglobin concentrations. The test results may differ at given concentrations due to individual gradation ranges of the tests (ref. to Table 2).

The results of the pathological erythrocyte readings and the hemoglobin reading differ with some manufacturers. The best performance for erythrocytes and hemoglobin readings was shown by the Combur-Test urine test strip. One reason for the difference is that the concentration ranges of some manufacturers are not identical. Furthermore it is more difficult to assign visual readings of intact erythrocytes to the appropriate concentration range. Especially in the lowest gradation range, few erythrocytes are already reported as positive. This could be clearly demonstrated with the Arkray Aution strip which reads negative with lysed erythrocytes at a concentration of 10/µL and positive with intact erythrocytes also with 10/µL despite the fact that a positive reading according to Table 2 should appear only with ≥20 erythrocytes/µL.

DISCUSSION

In 1992 Brigden at. al (4) published the results of a systematic analysis of the incidence of the presence of ascorbic acid in urine samples of a normal population. An examination of 4379 routine urinalysis specimens by Brigden found 22.8% positive for ascorbic acid at a mean concentration of 370 mg/l with a range of 70-3400 mg/l. In the same study, Brigden could show that even a moderate intake of 250 mg/day vitamin C can produce a mean ascorbic acid level of 310 mg/l, and a dose of 1500 mg/day a mean ascorbic acid level of 630 mg/l. Additional investigations of the effect of a controlled intake of ascorbic acid clearly confirmed that significant ascorbic acid levels are a common fact in routine urinalysis (5).

Brigden found that several urine test strips, except Chemstrip* urine test strips, yielded false-negative results with pathologic samples. (Chemstrip* is the US brand of the Combur-Test manufactured by Roche Diagnostics, formerly Boehringer Mannheim).

In the years since the publication of Brigden's study in 1992 the ingestion of ascorbic acid is playing an ever increasing role. The worldwide annual demand for ascorbic acid in 1995 was estimated at 60,000 tons – an increase by 50% since 1980 (6). One third of the total production is used for vitamin preparations in the pharmaceutical industry. The rest of 40,000 tons is mainly used as an additive by the food industry, and ascorbic acid is present in almost all kinds of packaged food and beverages as a preservative and for quality improvement. The additives are indicated on the packaging as E 300, E 301, E 302, E 303, or E 304. As a consequence and also considering the increasing trend to convenience food, the incidence of ascorbic acid concentrations ≥ 400 mg/L in urine samples must have increased significantly, with a potential impact on urinalysis dipstick readings. Roche Diagnostics is using iodate to widely eliminate the interference of ascorbic acid on their urine test strips (1, 5). New studies which could demonstrate whether other manufacturers have also adapted dipstick performance by reducing or eliminating the ascorbic acid interference were not available.

CONCLUSION

In routine urinalysis there is a tendency to reduce costs by streamlining laboratory procedures. Thus, normal urinalysis dipstick results would often not be followed by further urine examinations.

The findings of the present study demonstrate that if the urinary ascorbic acid content is not considered, most urinalysis dipsticks would even today report potentially dangerous false-negative results for glucose and hemoglobin. The approach to determine the ascorbic acid status of urine samples routinely in order to establish which adjustments need to be made does not eliminate the problem. A much better and cost-efficient proceeding is to use a test which is by design largely resistant to ascorbic acid.

Acknowledgements

We wish to thank Mrs. Isabel Heintz and Andrea Honecker for their technical assistance. The work was supported by Roche Diagnostics GmbH, Mannheim, Germany.

References:

1. Kutter D. Schnelltests in der klinischen Diagnostik. Urban & Schwarzenberg. München-Wien-Baltimore 1983; pp 33-34
2. Zweig MH, Jackson A. Ascorbic acid interference in reagent-strip reactions for assay of urinary glucose and hemoglobin. Clin Chem 1986;32:674-677
3. Berg B. Ascorbate interference in the estimation of urinary glucose by test strips. J Clin Chem Clin Biochem 1986; 24:89-96
4. Brigden ML, Edgell D, McPerson M, Leadbeater A, Hoag G. High incidence of significant urinary ascorbic acid concentrations in a west coast population – implications for routine analysis. Clin Chem 1991; 38: 426 – 431
5. Nagel D, Seiler D, Appel W, Rodrian J, Tritschler W. Einfluß von Ascorbinsäure auf einen neuen Harnteststreifen zum Nachweis von Erythrozyten. Das Medizinische Laboratorium 1982; 35: 137 –140
6. Elvers B, Hawkinds S (Eds.), Ullmann´s Encyclopedia of Industrial Chemistry, 5th Completely Revised Edition, Volume A27, Page 559, VCH 1996

Correspondence: Dr. Dietmar Nagel
Department of Clinical Chemistry
Klinikum der Stadt Ludwigshafen gem. GmbH
Bremserstrasse 79
D-67063 Ludwigshafen am Rhein
Germany
Telefon: +49-(0)6 21-5 03 35 52
Fax:+49-(0)6 21-5 03 35 55
e-mail: nageld@klilu.de