The Basic Analytical Performance of the Fully Automated Integrated Urine Analyzer UX-2000 CHM Unit

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In this report, we have examined the basic analytical performance of UX-2000 CHM (urine chemistry) unit. These results will be helpful for individual customers considering how to integrate this instrument into their existing urinalysis workflow. We examined correlation, sensitivity, sample carry-over, influence of interfering substance, and open vial stability of the test strip. The results of correlation, sensitivity, carry-over were favorable. We confirmed the influence of 60-100 mg/dl of ascorbic acid on BIL, BLD and NIT, which utilize an oxidation-reduction reaction as a principle. Moreover, the test strip stability of 12 days when stored in the bottle with drying agent was satisfactory. We were able to clarify the basic analytical performance of UX-2000 in various evaluations. As we could see in the evaluation results, we often face the discrepancies between the result of the test strip and result of the urine particle analysis. In such a case, it is possible to detect the discrepancy by utilizing the cross-check function installed in the UX-2000. Proper understanding of the basic analytical performance of the instrument helps operators with the use and result interpretation, and we believe that it may provide data outcomes with higher precision.

Key Words Fully Automated Integrated Urine Analyzer, UX-2000, Test Strip Analysis, Urine Particle Analysis, Ascorbic Acid, Crosscheck

INTRODUCTION

Despite being noninvasive, urinalysis provides a great deal of information and plays an important role in the diagnosis of kidney and urological diseases and in monitoring the effectiveness of treatment of chronic diseases. The clinical usefulness of urinalysis in screening and diagnosis is mentioned in the clinical guidelines on kidney and urinary tract diseases, and the results of urine test strip analysis and urine sediment analysis are considered crucial for understanding the pathophysiology. In short, to detect kidney and urinary tract diseases and understand the pathophysiology, it is necessary to conduct urinalysis such as test strip, urine particle analysis and urine sediment analysis accurately and to assess their results in a comprehensive manner. The Guidelines for Diagnosis of Hematuria [1)] specifies assessment of hematuria on the basis of urine occult blood reaction and urine red blood cell count. The Clinical Practice Guidebook for Diagnosis and Treatment of Chronic Kidney Disease 2012 [2)] specifies defining chronic kidney disease (CKD) based on signs that suggest kidney disorder, such as macro albuminuria, micro albuminuria, and hematuria.

The automation of urinalysis, as represented by tests conducted with urine test strip analyzers and urine particle analyzers, has advanced against this background as a rapid means of generating highly accurate information for screening [3)]. Wider use of automated analyzers has realized increasingly rapid and efficient urinalysis. Apart from that, there are great expectations from automated analyzers as sources of better quality data and highly useful clinical information.

The fully automated integrated urine analyzer UX-2000 by Sysmex (hereinafter UX-2000) is an analyzer that integrates urine test strip analysis with urine particle

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analysis based on flow cytometry (FCM). By combining these two functions, not only has the analyzer become more compact and user friendly but it has also become possible to manage the results of these two types of tests in a unified manner [4]. In other words, because the analyzer can rapidly provide highly accurate and comprehensive urinalysis results, it has now become possible to more accurately understand the pathophysiology-related information.
Both urine test strip analysis and urine particle analysis have their advantages and disadvantages. Test strip analysis can detect multiple parameters in one go but are prone to the effects of interfering substances present in the urine, which could give rise to false positive or false negative results. The FCM-based urine particle analysis can accurately count particles like red blood cells, white blood cells, bacteria, etc. But if there is hemolysis, disintegration or morphological changes in the particles, it cannot sometimes detect these components accurately. Thus, we can obtain highly accurate test results by using the two methods to complement each other while keeping in mind the advantages and disadvantages of each method. To effectively operate a UX-2000 according to the situation in a clinical laboratory. First we have to have a clear understanding of the analyzer's performance with objective data. In the present study, we evaluated the basic performance of UX-2000 in urine test strip analysis in order to maximize its performance, and we report the results here.

SPECIMENS AND ANALYZER USED

1. Specimens

Patients' urine specimens submitted to the Department of Central Clinical Laboratory, Meijo Hospital during June-July 2011 and pooled negative urine specimens were used to prepare test samples to which various reagents were added.

2. Analyzer and reagents

Urine test strip analysis, urine particle analysis: Meditape II 10U, UX-2000
Urine biochemistry analysis (quantitative)
Glucose: Quick Auto Neo GLU-HK
(hexokinase ultraviolet assay, Shino-test)
Creatinine: Pure Auto S CRE-L
(enzymatic method, Sekisui Medical)

3. Table of abbreviations

As many abbreviations are used in this document, we have listed them in a table (***Table 1***).

Table 1 *Abbreviations*

	Abbreviations	Item
Urine strip test	GLU	Glucose
	PRO	Protein
	BIL	Bilirubin
	CRE	Creatinine
	BLD	Blood
	KET	Ketones
	NIT	Nitrite
	LEU	Leukocytes
	P/C	Protein/Creatinine ratio
Urine particle analysis	RBC	Red blood cells
	WBC	White blood cells
	EC	Epithelial cells
	CAST	Casts
	BACT	Bacteria

METHODS

1. Tests on correlation

1) Correlation between urine test strip and urine biochemistry test results

The urine specimens of the same patients were analyzed by the UX-2000 and through urine biochemistry analysis, and the correlation between the test strip and biochemistry test results for GLU (n = 291) and CRE (n = 284) was examined.

2) Comparison between urine test strip and FCM results

The urine specimens of patients were analyzed using UX-2000, and results for BLD and RBC (n = 1,242) and LEU and WBC (n = 1,243) respectively obtained by the test strip and FCM were compared. Various indices were calculated from the analysis results using the formulas shown in ***Table 2***.

2. Sensitivity tests

1) Evaluation using patient urine specimens

A hematuria specimen (RBC: 5,123/μL) and a pyuria specimen (WBC: 5,611/μL) were diluted with pooled negative urine to adjust the concentration on the basis of FCM so that BLD and LEU were near their detection limits (***Table 3***). Three replicate measurements were made with the UX-2000 on the samples thus prepared and the detection sensitivity for BLD and LEU evaluated.

2) Evaluation using samples having added reagents

Various reagents were added to pooled negative urine to prepare samples having GLU, PRO, BIL, CRE, BLD, KET and NIT near their respective detection limits (***Table 3***). The reagents used for this purpose were D-(+)-glucose (guaranteed reagent, Nacalai Tesque), TP standard solution (Sysmex), bilirubin standard solution-E (Sysmex), creatinine (Tokyo Chemical Industry), hemoglobin S (SIGMA), lithium acetoacetate (Tokyo Chemical Industry) and sodium nitrite (guaranteed reagent, Wako Pure Chemical Industries). Three replicate measurements were made with each sample using UX-2000.

3. Tests on carryover

1) Evaluation using patient urine specimens

Three replicate test strip measurements were performed on pooled negative urine samples with the UX-2000. Similarly, three replicate test strip measurements were performed with highly concentrated patient urine samples having hematuria (RBC: 5,386/μL), pyuria (WBC: 5,563/μL) or bacteriuria (BACT: 10,956/μL). Then three replicate measurements were again performed with the pooled negative urine to evaluate carryover of BLD, NIT and LEU.

2) Evaluation using specimens having added reagents

Highly concentrated samples were prepared by adding different reagents to pooled negative urine (GLU: 1,500 mg/dL, PRO: 1,000 mg/dL, BIL: 15 mg/dL, CRE: 500 mg/dL, BLD: 2.0 mg/dL, KET: 200 mg/dL, NIT: 0.5 mg/dL), and the carryover of each substance was evaluated by the same procedure as in 3.1).

Table 2 *Calculation formulas*

Sensitivity	(The number of positive samples in urine strip test/The number of positive samples in FCM analysis) × 100
Specificity	(The number of negative samples in urine strip test/The number of negative samples in FCM analysis) × 100
Concordance	(The number of negative concordant samples + The number of positive concordant samples)/n × 100
Miss rate	1 - Sensitivity
Positive predictive rate	(The number of positive samples in FCM analysis/The number of positive samples in urine strip test) × 100
Negative predictive rate	(The number of negative samples in FCM analysis/The number of negative samples in urine strip test) × 100

Table 3 *Theoretical detection sensitivity of strip test urine parameters* [5)]

Item	Min. detecting sensitivity
GLU	30mg/dL
PRO	10mg/dL
BIL	0.5mg/dL
CRE	10mg/dL
BLD	0.03mg/dL
	Approx. 10/μL
KET	Acetoacetic acid 5mg/dL
NIT	0.08mg/dL
LEU	Approx. 25/μL

4. Effect of interfering substances

1) Ascorbic acid

A hematuria specimen (RBC: 5,123/μL) was diluted with pooled negative urine, and samples with different RBC concentrations (40, 58 and 161/μL) were prepared on the basis of FCM. Separately, various regents were added to pooled negative urine to prepare samples having different concentrations of GLU (50, 100 and 150 mg/dL), BIL (0.5, 2.0 and 10.0 mg/dL), BLD (Hb: 0.03, 0.10 and 0.50 mg/dL), and NIT (0.15 and 0.45 mg/dL). Ascorbic acid was added to different concentrations (0, 20, 40, 60, 80, 100 and 150 mg/dL) to these samples, and its effect on GLU, BIL, BLD and NIT measurements by UX-2000 was evaluated.

2) High concentrations of glucose

A pyuria specimen (WBC: 5,611/μL) was diluted with pooled negative urine and samples with different adjusted WBC concentrations (15, 23, 46 and 92/μL) were prepared on the basis of FCM measurements. Glucose was added to different concentrations (1,000, 2,000, 3,000 and 3,500 mg/dL) and the samples analyzed by the UX-2000 to evaluate the effect of high glucose concentrations on LEU analysis.

5. Tests of open-package stability of test strips

The control substances (Meditape Check 1 and 2, Sysmex) of the same lot were analyzed on alternate days during a 12-day period by the UX-2000 using test strips from the same bottle. The strips were stored after bottle opening under 3 different conditions, namely, 1) in a bottle containing a drying agent, 2) in the feeder of the analyzer, and 3) in a bottle without a drying agent, and the stability of the test strips for different parameters was evaluated.

RESULTS

1. Tests on correlations

1) Correlation between urine test strip and biochemistry test results

For GLU, there was exact concordance in 97.3%, and within ± 1 grade in 99.7% cases (***Table 4-a***). For CRE, there was exact concordance in 77.1% and concordance within ± 1 grade in 98.9% cases (***Table 4-b***).

Table 4 *Correlation between urine strip test results and quantitative urine biochemistry test results*

a. GLU

: Exact concordance : Concordance within ±1 grade

Urine strip			Biochemistry test (mg/dL)										
	Qualitative value	Semi-quantitative value	–	30	50	70	100	150	200	300	500	1000	OVER
UX-2000	–		260	2									
	±	30	1	1	1	1							
		50		1	5								
	1+	70				2							
		100											
	2+	150						2					
		200							2				
	3+	300								3			
		500									5		
	4+	1000										1	
		OVER										2	2

Exact concordance 97.3% n=291
Concordance within ±1 grade 99.7%

Qualitative value	Semi-quantitative value	Range of biochemistry test results (mg/dL)
–		-29
±	30	30-39
	50	40-59
1+	70	60-84
	100	85-124
2+	150	125-174
	200	175-249
3+	300	250-399
	500	400-749
4+	1000	750-1499
	OVER	1500-

b. CRE

Urine strip		Biochemistry test (mg/dL)					
	Semi-quantitative value	10	50	100	200	300	OVER
UX-2000	10	19					
	50	15	79	20	2		
	100		9	61	5		
	200		1	22	33	2	
	300				8	9	
	OVER						1

Exact concordance 71.1% n =284
Concordance within ±1 grade 98.9%

Semi-quantitative value	Range of biochemistry test results (mg/dL)
10	-29
50	30-74
100	75-149
200	150-249
300	250-399
OVER	400-

2) Comparison between urine test strip and FCM results
When samples with BLD 1 + or more and RBC 27.8/μL (5/HPF) or more are defined as positive, the sensitivity was 86.2%, specificity 88.5%, concordance 88.1%, and miss rate 13.8% (***Table 5-a***). When samples with LEU 75/μL or more and WBC 27.8/μL or more are defined as positive, the sensitivity was 75.5%, specificity 96.5%, concordance 92.8%, and miss rate 24.5% (***Table 5-b***).

2. Sensitivity tests

When the concentration that gave the qualitative result of ± or more is defined as the detection sensitivity, the sensitivity was 30 mg/dL of glucose for GLU, 15 mg/dL of albumin for PRO, 0.35 mg/dL of direct bilirubin for BIL, 25.2/μL of RBC and 0.03 mg/dL of Hb for BLD, 4 mg/dL of lithium acetoacetate for KET, 0.075 mg/dL of sodium nitrite for NIT, and 8.8/μL of WBC for LEU. The result of CRE is displayed as not a qualitative but semi-quantitative, and its result agreed with the theoretical concentration of creatinine (***Table 6***).

Table 5 *Comparison of urine strip test results and urine particle analysis results*

a. Urine red blood cells n =1242

		FCM results RBC (#/μL)							
		<5.6 (1</HPF)	<27.8 (1-4/HPF)	<55.6 (5-9/HPF)	<111.2 (10-19/HPF)	<166.8 (20-29/HPF)	<222.4 (30-49/HPF)	<566.0 (50-99/HPF)	≧566.0 (>100/HPF)
Urine strip BLD	−	472	266	14	5	0	0	0	0
	±	33	123	9	4	0	0	0	0
	1 +	8	85	42	19	0	2	0	0
	2 +	0	17	22	20	10	5	6	0
	3 +	1	5	7	14	4	4	18	27

Sensitivity	86.2%
Specificity	88.5%
Concordance	88.1%
Miss rate	13.8%
Positive predictive rate	63.3%
Negative predictive rate	96.5%

Negative
Positive

b. Urine white blood cells n =1243

		FCM results WBC (#/μL)							
		<5.6 (1</HPF)	<27.8 (1-4/HPF)	<55.6 (5-9/HPF)	<111.2 (10-19/HPF)	<166.8 (20-29/HPF)	<222.4 (30-49/HPF)	<566.0 (50-99/HPF)	≧566.0 (>100/HPF)
Urine strip LEU	−	696	248	21	2	0	2	0	0
	25 / μL	7	40	13	10	4	0	1	0
	75 / μL	2	23	15	16	3	3	2	0
	250 / μL	0	11	6	11	5	7	16	1
	500 / μL	0	0	5	7	6	6	17	37

Sensitivity	75.5%
Specificity	96.5%
Concordance	92.8%
Miss rate	24.5%
Positive predictive rate	81.9%
Negative predictive rate	94.9%

Negative
Positive

Table 6 *Results of sensitivity tests on urine strip test parameters*

Item	Concentration	Measurement		
		1	2	3
GLU (mg/dL)	15	-	-	-
	30	±	±	±
	45	±	±	±
PRO (mg/dL)	5	-	-	-
	10	±	-	-
	15	±	±	±
	25	1+	1+	1+
BIL (mg/dL)	0.20	-	-	-
	0.35	1+	1+	1+
	0.50	1+	1+	1+
RBC (#/μL)	11.2	-	-	-
	15.5	-	-	-
	17.5	-	-	-
	25.2	-	-	±
	29.3	±	±	±
	40.1	±	±	±

Item	Concentration	Measurement		
		1	2	3
Hb (mg/dL)	0.02	-	-	-
	0.03	±	±	±
KET (mg/dL)	3.0	-	-	-
	4.0	±	±	±
NIT (mg/dL)	0.050	-	-	-
	0.075	1+	1+	1+
	0.100	1+	1+	1+
WBC (#/μL)	6.4	-	-	-
	8.8	25	25	-
	17.2	75	75	75
CRE (mg/dL)	10	10	10	10
	30	10	10	10
	40	10	10	10
	50	50	50	50

3. Tests on carryover

No carryover was detected for any of the parameters.

4. Effect of interfering substances

1) Ascorbic acid

The ascorbic acid concentration at which the qualitative value of 1 + turned negative was 40 mg/dL for BLD, 60 mg/dL for GLU, and 100 mg/dL for BIL and NIT (***Fig. 1-a*** to ***-e***).

2) High concentrations of glucose

The concentration of glucose that turned the semi-quantitative value of 25/μL of LEU into negative was 2,000 mg/dL. Although the glucose concentration of 2,000 mg/dL made the semi-quantitative value of LEU 75/μL one grade lower, LEU did not become negative even when the glucose concentration was raised to 3,500 mg/dL (***Fig. 1-f***).

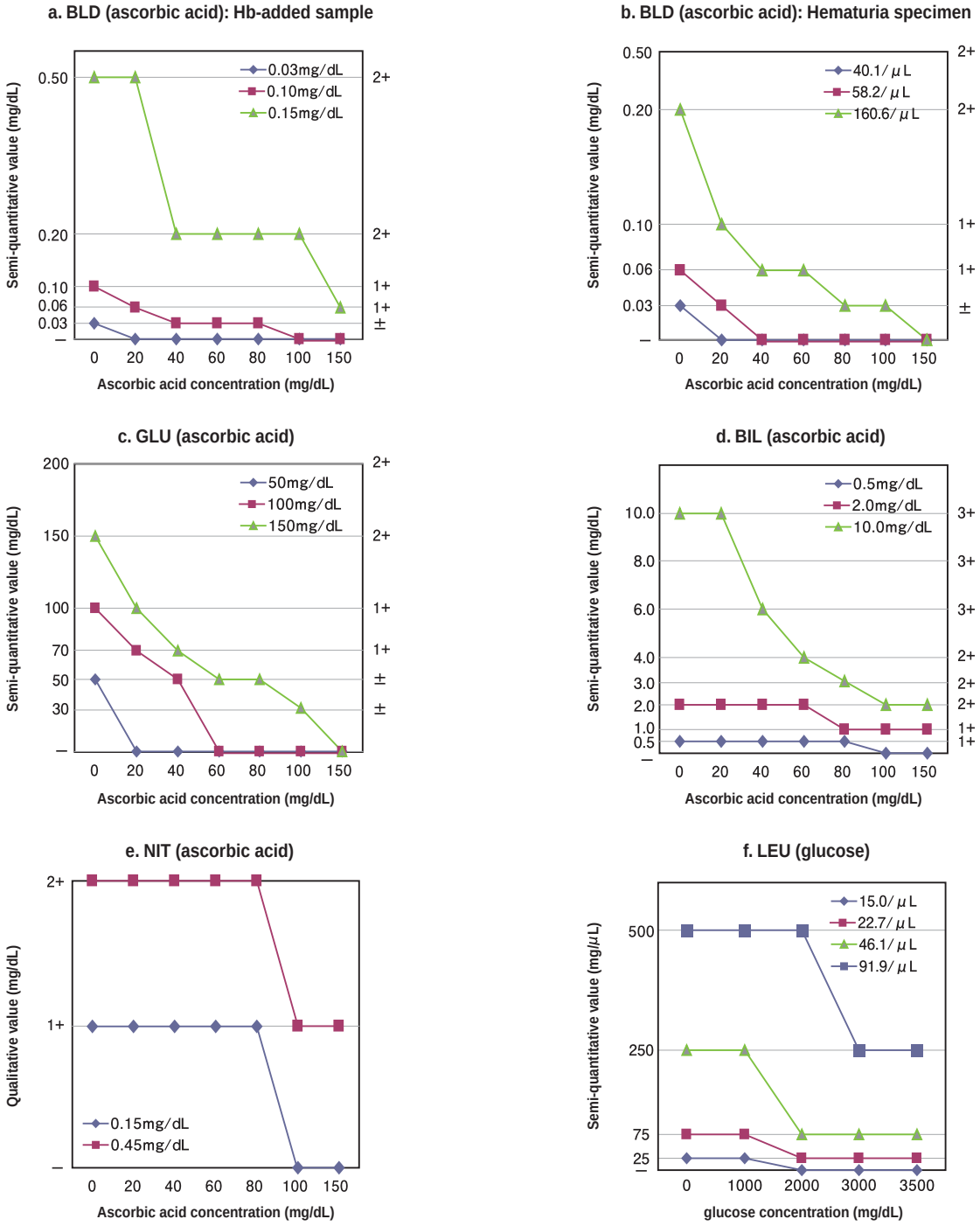

Fig. 1 *Effect of interfering substances*

5. Tests of open-package stability of test strips

When test strips were placed in a bottle containing a drying agent (Condition 1), the qualitative value of LEU increased by 1 grade from day 6, but on day 12 it returned to the value of day 1. With other parameters, there was no change in the qualitative or semi-quantitative values up to day 12 from opening-package. When the test strips were kept in the feeder of the analyzer (Condition 2), from day 2 there was a reduction in the qualitative value of KET by one grade. With LEU, the qualitative value increased by one grade from day 4 onwards. When the test strips were stored in a bottle without a drying agent (Condition 3), the qualitative value of KET had increased by one grade on day 4, but the value of the first day was seen again on day 12. With LEU, the qualitative value had increased by one grade from day 8 but it returned to the first day's value on day 12 (***Fig. 2***).

DISCUSSION

Correlation between urine test strip and urine biochemistry test results

The concordance between the results of the two tests was good for GLU, which confirmed the high accuracy of the test strip analysis. In the test results evaluated here, there was one sample that did not fall within ± 1 grade concordance. This sample showed a lower value in the test strip analysis compared to the quantitative biochemistry test. This was suspected to be because of the effect of interfering substances like ascorbic acid. In the correlation of CRE with the biochemical test, the percentage of samples with exact concordance was slightly low, but the concordance was good within ± 1 grade. The low level of exact concordance was because of scatter of values within ± 1 grade at around the 100 mg/dL level. However, the reasons for greater variation in this range compared to other ranges were not clear.

We could thus confirm the accuracy of the urine test strip results through the evaluation conducted in the present study. Nevertheless, many of the urine specimens from patients used for the evaluation of GLU had glucose within the normal range. Therefore, for verifying the correlation in the high GLU concentration range, we need to further evaluate using a greater number of GLU positive samples.

Comparison between urine test strip and FCM results

The sensitivity for BLD, with reference to the FCM results, was acceptable at 86.2%. On the other hand, as the miss rate was 13.8%, caution should be demanded with patients whose blood cell count in urine is being monitored. In our evaluation, the LEU tended to be judged as negative with some WBC positive specimens, although the results of sensitivity tests were good. On the other hand, there is the possibility that the urine had white blood cells like lymphocytes in large numbers, or the results were affected by an interfering substance in the urine because of antibiotic use [6]. It is useful to take FCM results also into account when inflammation in urinary tract is suspected.

Sensitivity tests

In general, concentrations around the theoretical detection limits could be detected for all the test strip parameters, which represented good detection sensitivity. However, with BLD, the sensitivity was low as the measured values were lower than the theoretical RBC concentrations. On the other hand, good sensitivity was shown when hemoglobin-added samples were evaluated. Therefore, it is possible that disruption of red blood cell membranes (hemolysis) when the red blood cells came into contact with the reagent pad was insufficient in hematuria specimens.

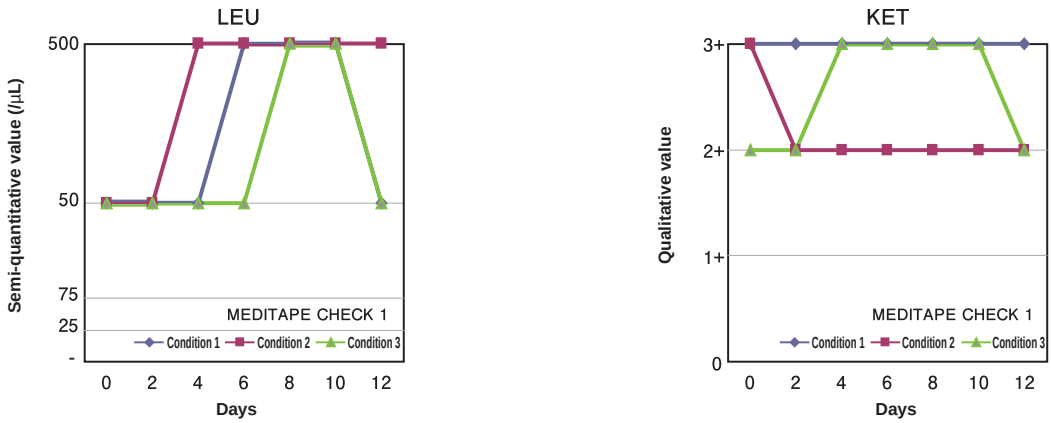

Fig. 2 *Stability of seal-opened test strips*

Effect of interfering substances - Ascorbic acid

Ascorbic acid is known to cause a false negative reaction in the measurement of test strip parameters like BLD and GLU, which are based on oxidation-reduction reaction [6]. In the evaluation of interfering substances in the present study, BLD was affected from 40 mg/dL of ascorbic acid upwards, GLU from 60 mg/dL, and BIL and NIT from 100 mg/dL. This confirms the possibility of false negative results under high ascorbic acid concentration in the sample (40-100 mg/dL). According to one report, 50 mg/dL or more of ascorbic acid is found in about 5% of patient urine specimens [7]. Therefore, if intake of ascorbic acid is suspected from patient interviews, etc. FCM measurement also for detection of red blood cells and bacteria would be useful.

Effect of interfering substances - High concentrations of glucose

There have been reports of false negative LEU results in urine test strip analysis due to the presence of a high concentration (3,000 mg/dL) of glucose [7]. In our evaluation also, false negative results were shown from glucose concentration 2,000 mg/dL upwards. Therefore, if GLU is strongly positive in the urine test strip, it is better to take the caution regarding its possible interference into account in LEU measurements.

Open-package stability of test strips

It is known that urine test strips degenerate when exposed to high temperature and high humidity [8]. Therefore, we evaluated the open-package stability of test strips, stored under different conditions, in the various parameters. The strips remained more or less stable for 12 days under all the storage conditions tested. A change of one grade was seen with KET, LEU, etc. but there were no definite trends and the cause seemed to be variation in the result of measurement around the boundary between two grades. Nevertheless, the urine test strips degenerate if exposed to direct sunlight or humidity even for a short time [8]. Therefore it is important to ensure a proper storage environment for them to obtain correct measurement results. It is preferable to return test strips to their bottles for storage when they are not in use.

In the present study we evaluated the basic performance of UX-2000 in test strip analysis. Understanding the basic performance of the analyzer is helpful for appropriate data interpretation, and can contribute to providing of highly accurate test data. We found that sometimes there is discrepancy between the results of urine test strip and urine particle analysis because of differences of the measurement principle. The establishment of cross-check function of UX-2000 can be used to identify the cases showing discrepancy between BLD and RBC, LEU and WBC, and NIT and BACT and improve accuracy of the screening. In short, we can provide high quality data by understanding the characteristic features of each measurement and comprehensively interpreting urinalysis results obtained by the two methods.

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