TESTING OF IQ™ 200 AUTOMATED URINE ANALYZER ANALYTICAL PERFORMANCES IN COMPARISON WITH MANUAL TECHNIQUES

ISPITIVANJE ANALITIČKIH KARAKTERISTIKA IQ™ 200 AUTOMATSKOG ANALIZATORA ZA URINE U POREĐENJU SA MANUELNIM TEHNIKAMA

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Summary: Automation is necessary in laboratory systems. It enables reduction of time required for sample analysis, as well as standardization of methods. However, automation of urine control in laboratories is much less common than in hematological analyses. Not long ago, the necessary automated systems for urine analysis have also been developed. The objective of this study is a comparison of the IQ™ 200 automated system for urine analyzing with standardized manual urine analyzing techniques. Comparative analysis of 300 samples was performed by the IQ™ 200 system and by the standardized methods of manual microscopy and chemical urine analysis. The results acquired point to very high compatibility between urine analyses by manual techniques and by the automated system IQ™ 200, and in some analyses IQ™ 200 showed higher sensitivity. It can be concluded, with the aim of standardization and shortening of time required for urine analysis, that utilization of automated urine analyzing systems is recommendable, especially in institutions with a large number of daily analyses. This is also supported by the fact that operation procedure on automated systems is much more simple in comparison to manual techniques.

Keywords: automated urine analysis, urine sediment, chemical urine analysis

Kratak sadržaj: Automatizacija je neophodna u laboratorijskim sistemima. Njome se postiže skraćenje vremena neophodnog za analiziranje uzoraka, kao i standardizacija metoda. Međutim, u laboratorijama je automatizacija u pregledu urina u mnogo manjoj meri zastupljena u odnosu na hematološke analize. Tek relativno nedavno razvijeni su potrebni automatizovani sistemi i za analizu urina. Cilj ovog rada je bio da se izvrši upoređivanje automatizovanog sistema za analizu urina IQ™ 200 sa standardizovanim manuelnim tehnikama analiziranja urina. Izvršeno je uporedno ispitivanje 300 uzoraka urina na sistemu IQ™ 200, i standardizovanim metodama manuelne mikroskopije i hemijskog pregleda urina. Dobijeni rezultati ukazuju na veoma visoku podudarnost analize urina manuelnim tehnikama i automatizovanim sistemom IQ™ 200, pri čemu je kod nekih pretraga IQ™ 200 pokazao veću osetljivost. Može se zaključiti da je, u cilju standardizacije i skraćenja vremena analize urina, preporučljiva primena automatizovanih sistema za analiziranje urina, naročito u ustanovama sa velikim brojem dnevnih analiza. Tome u prilog ide i činjenica da je postupak rada na automatizovanim sistemima daleko jednostavniji u odnosu na manuelne tehnike.

Ključne reči: automatizovana analiza urina, sediment urina, hemijski pregled urina

Introduction

Urine analysis is one of the most frequently utilized analyses in the routine laboratory practice. It is performed in order to diagnose diseases, monitor their progression and efficiency or complications during therapy, as well as to screen the non-symptomatic population for congenital or hereditary diseases (1).

Urine analysis should be based on standardized procedures of sample gathering, transporting and analyzing. Within the scope of standardization of these procedures, there is a necessity of results interpretation correlation and referent ranges correlation, and for complete successfulness, maximum reduction of subjectivity of the analyst is also necessary. To improve standardization, the European Confederation of Laboratory Medicine has already in 2000 published Guidelines for Manual Counting of Particles in Non-Centrifuged and Concentrated Urine Samples

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(2). However, the need for updating this diagnostic procedure and utilization of automation as a basic conception of laboratory practice resulted in the development of first automated systems for urine analysis. From the moment of emergence of the first automated urine analyzers, such as Yellow IRIS, with its operation based upon the principle of microscopic particle identification (3), then Sysmex UA series based upon the principle of effluent cytometry (4) and other systems based upon the principle of effluent cytometry (5), newer and more modern urine analyzers have been developed. Amongst them is the IQ™ 200 automated urine analyzing system. This system has incorporated the IQ™ 200 automated microscopic urine analyzer and AUTION MAX AX-4280 automated chemical urine analyzer, in order to obtain complete integration of microscopic and chemical urine analyses.

The objective of this paper is to perform the analytical evaluation of IQ™ 200 system in relation to the standardized microscopic urine sediment analysis, as well as the standardized chemical urine analysis.

Materials and Methods

By the IQ™ 200 system (IRIS, USA and ARKRAY, Japan) and the standard procedures of manual microscopy and chemical urine analysis (by Multistix sticks for urine chemical analysis), 300 samples, received in the Laboratory for Renal Pathological Physiology, Clinical Center of Vojvodina in Novi Sad in the period between 7–11 of July 2008, were analyzed

comparatively. The samples were analyzed not later than two hours after receipt. For analysis performed by the IQ™ 200 system, 4 mL of native, non-centrifuged urine was sufficient.

The urine samples were first analyzed by the IQ™ 200 system, and then the chemical analysis was performed by a standardized manual procedure, after which the urine samples were centrifuged and prepared by standardized procedure for manual microscopy. All elements of chemical examination were compared, such as: glucose, proteins, bilirubin, urobilinogen, pH, ketones, nitrites, pus (leukocytes), specific gravity and blood (hemoglobin). The only difference in the report was the following one: on standardized sticks for chemical analysis (Multistix, Bayer, Germany) the name of an item was hemoglobin, while in the IQ™ 200 system, the same item was indicated as blood. Furthermore, the automated system chemical analysis reports contain items like color and appearance, readout of which is performed with the naked eye in the manual procedure. Certain elements of urine sediment separated by manual microscopy were also compared to those analyzed by the IQ200 system: erythrocytes, leukocytes, crystals and cylinders. The samples containing on average 1–3 erythrocytes and up to 5 leukocytes in one field of view (frame) were considered as regular findings by manual microscopy.

Chemical analysis by manual procedure was performed with Multistix sticks, and the urine samples were poured into plastic test tubes and prepared for microscopic analysis as per the following conditions:
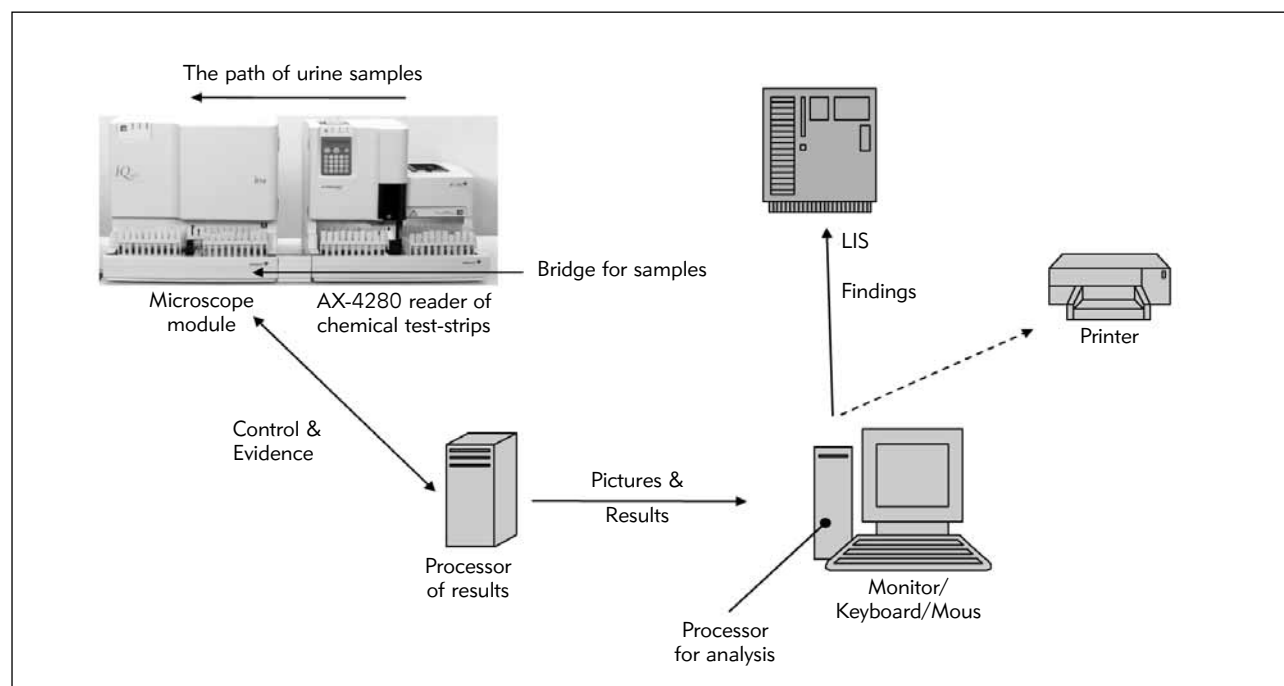

Figure 1 IQ™ 200 – Architecture of the system (combination of IQ200 and AX-4280 analyzers).

a) urine volume –10 mL

b) centrifuging period – 5 minutes

c) centrifuging velocity – 4000 r/min

d) volume of urine sediment – 0.5 mL

e) volume of urine sediment for microscopy – 10 mL

f) covering glass – 18 x 18 mm.

Ten frames with high magnification (400) were examined, after which the medium value of particles found per one frame was calculated. This value was then compared to the results obtained by the IQ™ 200 system, by comparing whether the increase of a certain element in a sediment analyzed by the manual method (positive result) corresponds to any increase of the same element analyzed by the IQ™ 200 system. Although there is a formula for conversion of the number of cells in one frame to the number of cells in mL (6), it was not used in this study.

IQ™ 200 *Urine analyzing system*

IQ™ 200 is a completely automated urine analyzing system. It is composed of 4 modules, interconnected with communication cables (*Figure 1*):

• IQ200 microscope module

• Processor for analysis with monitor, keyboard and mouse

• Processor for results

• AX-4280 chemical module.

The IQ200 can also be utilized as an independent unit for microscopic analyses of urine sediments, or in combination with a chemical module.

The IQ200 microscope module automatically identifies and processes the samples in test tubes and stands, having 10 positions (IQ200 Automated Urine Microscopy Analyzer Operators manual-Rev C 06/2004). The stands with test tubes can be positioned directly in the IQ200 microscope analyzer, or they can be transferred automatically from AX-4280 across the bridge connecting the chemical and microscope component of the system. The microscope analyzer is composed of a microscope, a digital camera CCD and a flow cell. The aspirated sample (aspiration volume is 0.9 mL) is represented in the microscope objective, where this sample is surrounded by lamina. Lamina is an isotonic solution containing appropriate flow stabilizers, bacteriostatic and fungicidal ingredients and stabilizers. Its role is to provide a laminar, uniform flow of a sample and to enable the optimal position of all sample particles for their further analysis. CCD digital camera is connected to the microscope, shooting 500 shots per one sample, with regard to the fact that each field of vision is alighted by a stroboscopic light bulb (*Figure 2*).

The resulting shots are digitalized and transferred into a computer for analysis (AP-analysis processor). Shots of certain particles are distinguished in each picture and by the APR – auto particle recognition program, which is practically a neural network programmed for recognition of urine elements, the characteristics of each element can be recognized. Those specific characteristics analyzed for each isolated element are: size, contrast, shape and structure, whereby each characteristic is analyzed by a series of specific algorithms transformed into numerical values. In case the identification probability for a certain element is below 90% accuracy, that element is placed in the unclassified category. Otherwise, the recognized elements are classified in 12 categories: erythrocytes, leukocytes, clusters of leukocytes, hyaline cylinders, unclassified cylinders, laminated epithelial cells, other epithelial cells, bacteria, fungi, crystals, mucus and sperm. Particles sized below 3 mm are classified as »total small particles« and positioned on the screen as number of particles/mL. By prolonging the sampling time prior to analysis, this number increases. Concentration of particles is calculated based upon the number of shots and sample volume.

Quality control of analyzer's performance is carried out by monthly calibration of the analyzer, and then by positive (803–1203 particles/mL) and negative control (0–20 particles/mL), on a daily basis, prior to urine sample analysis. Each day, but also after analyzing highly concentrated samples, control of sharpness of the flow cell central part (focus) to which the microscope is directed is also necessary.
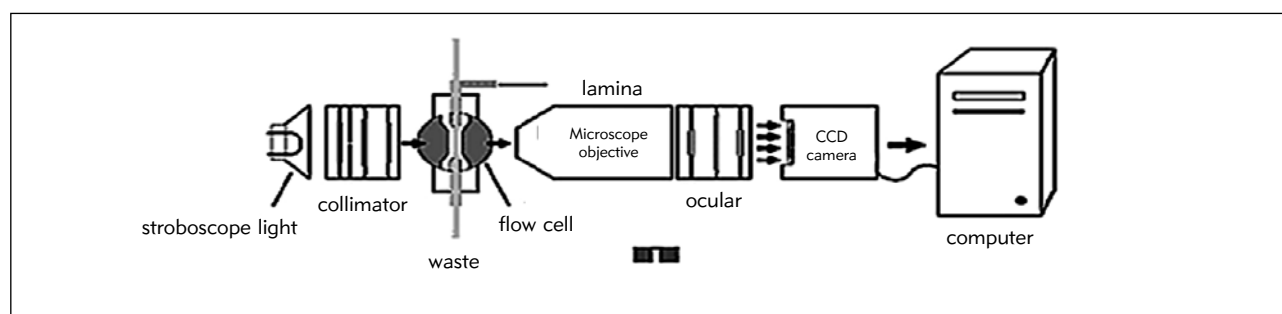

Figure 2 Digital shooting and analysis of pictures in flow cell (method of analysis of microscope module).

AX-4280 is a chemical module for urine analysis in the scope of IQ™ 200 system, which is extremely simple to use because, after positioning the sample into the stand, it is only required to insert the sticks in the drum of the instrument, and press the START button (Aution Max-4280 operating manual, Arkray, Kyoto, Japan). The pipette aspirates the sample, agitates it and automatically distributes the drops on the test sticks. Capacity of the cylinder is 200 sticks, however, it is recommended to insert the number of sticks that corresponds to the required number of analyses. The only sticks that can be utilized are Aution sticks (Arkray, the Netherlands) packed by 100 pieces. These sticks do not contain the vitamin C option, thus, in order to evidence possible faulty results of erythrocyturia and leukocyturia, which is the most frequent interference, apart from these sticks, the sticks containing this option are also necessary. Minimum volume of urine required for chemical analysis is 2 mL (aspirating volume is 0.95 mL), but due to its passing through the connecting bridge to reach the microscope module, pouring minimum 4 mL of urine is recommended. The measurement methods are the following: Test Strip–Dual (single) Wavelength Reflectance Method, Specific Gravity-Refractive Index Method, Color-Four Wavelength Reflectance Method, and Turbidity-Transparency Index Method. Quality control is performed monthly, with two control sticks in the range of high and low values.

Analytic evaluation of IQ200 microscope analyzer

For an analytical evaluation of the IQ™ 200 system microscope module, the following tests were performed: inaccuracy test in series, »day-after-day« inaccuracy, (reproducibility), accuracy and linearity, by using erythrocyte suspension in various concentration ranges.

Statistical methods

Statistical analysis, descriptive statistics and coefficient of correlation (7), were performed using Microsoft Office Excel program package 2003. Results are expressed as mean ($\bar{x}$) ± SD, and the coefficient of variation (CV) (8).

Results

Inaccuracy of the microscope module

Inaccuracy in series was determined in ten measurements of fixed erythrocytes suspension in clean solution as a negative control (0 particles/mL; part number 475–0058 lot. 344–07), and as a positive control (1000 particles/mL; part number 475–0046 lot. 344–07), which were delivered by the manufacturer, as well as of concentration range obtained by mixing equivalent parts of negative and positive controls (theoretical concentration 500 particles/mL).

A »day-after-day« inaccuracy measurement was carried out in the period of 10 days, also by using a negative (0 particles/mL) and a positive control (1000 particles/mL).

Inaccuracy of the microscope module is represented in *Table I*. The largest CV in both cases is in the low control range.

Table I Within-run and between-day imprecision for the microscopic analyzer Iris IQ200.

Declaration values (particles number/μL)	Within-run imprecision (n =10 samples)				Imprecision between-day (n =10 samples)			
	Range	$\bar{x}$	SD	CV %	Range	$\bar{x}$	SD	CV %
0	1–7	3.4	2.54	74.1	0–9	4	3.3	84.5
500	485–524	508.9	14.6	2.8	495–508	504.1	4.1	0.8
1000	904–1013	957.2	41.1	4.2	957–1086	1006.2	44.1	4.3

Table II Comparison between the number of erythrocytes and leukocytes in urine with manual and automatic microscopy.

Number of samples with:	Erythrocytes		Leukocytes	
	Manual microscopy	Automatic microscopy	Manual microscopy	Automatic microscopy
Registered presence of elements	255 (85 %)	290 (96.6 %)*	289 (96.3 %)	295 (98.3 %)
Pathological values	98 (32.6 %)	100 (33.3 %)	76 (25.3 %)	126 (42 %)**

Legend: *p<0.05, ** p<0.01

Linearity of the microscope module

Linearity was determined by using the original suspension of fixed erythrocytes in solution without any other particles. Linearity testing was carried out in five concentration ranges: 0, 250, 500, 750, 1000 particles/mL, prepared as follows: Negative control ready for application (part number 475–0058 lot. 344–07), number of particles = 0/μL; Concentration 1 (7.5 mL negative control and 2.5 mL positive control), number of particles = 250/μL; Concentration 2 (5 mL negative control and 5 mL positive control), number of particles = 500/μL; Concentration 3 (2.5 mL negative control and 7.5 mL positive control), number of particles = 750/μL; Positive control ready for application (part number 475–0046 lot. 344–07), number of particles = 1000/μL.

After testing 5 concentration ranges, the declared linearity limit for erythrocyte suspension up to 1000 particles/mL has been confirmed. The obtained values represented: for concentration 1 (250 particles /mL) 98% of theoretical values, for concentration 2 (500 particles/μL) 97.2% of theoretical values, for concentration 3 (750 particles/μL) 98.5% of theoretical values and for positive control 98.4% of the declared values.

Comparative testing of manual microscopy and the microscope analyzer IQ200

Erythrocytes and leukocytes

The results for erythrocytes and leukocytes are represented in *Table II*. By automated microscopy, the presence of erythrocytes and pathological values of leukocytes in urine were detected in a significantly higher percentage.

Cylinders and crystals

By manual microscopy, among 300 samples, the presence of crystals was detected in 26 samples and the presence of cylinders in 20 samples, while by automated microscopy the presence of crystals was detected in 20 samples and the presence of cylinders in 15 samples.

Comparative testing of chemical analyses carried out with Multistix sticks and with the chemical analyzer AX-4280

The results obtained by comparative testing of urine chemical analyses performed by Multistix sticks and by an AX-4280 analyzer are presented in *Table III*.

Except for nitrites, by the automated system chemical module some more positive findings were detected, although insignificantly, than by using Multistix sticks. By visual control of urine, in 176 samples the alteration of appearance was obvious, and AX-4280 demonstrated this alteration only in 65

Table III Urine chemical examination with Multistix sticks and AX-4280 chemical module of the system IQTM 200 – review of positive results.

Item	Multistix sticks	AX-4280
Glucose	11 (3.6 %)	22 (7.2 %)
Protein	104	112 (37.3 %)
Bilirubin	3 (1 %)	6 (2 %)
Urobilinogen	14 (4.6 %)	28 (9.3 %)
Nitrite	22 (7.2%)	9 (3 %)
Pus (Leukocytes)	61 (20.3 %)	71 (23.6 %)
Ketones	28 (9.3 %)	32 (10.6 %)
Blood (hemoglobin)	85 (28.3 %)	89 (29.6 %)

Table IV Comparison between pH and specific gravity using Multistix sticks and AX-4280 chemical module.

	Multistix ($\bar{x} \pm SD$)	AX-4280 ($\bar{x} \pm SD$)	Coefficient of correlation (r)
pH	5.6±0.81	5.92±0.71	0.63; p<0.001
Specific gravity	1022.5±6.1	1020.2±9.7	0.32; p<0.01

samples, while discrepancies in urine color determined by visual checkup and automated control occurred in 22 cases.

Results obtained by comparative testing of urine specific gravity and pH value are presented in *Table IV*. By comparison of the pH value results, higher correlation rate was obtained than in the case of specific gravity.

Discussion

In hospital institutions there is a frequent problem of prompt delivery of the first morning urine samples from certain clinics. Therefore, the recommended time of 2 hours (9), during which the urine analysis should be carried out, is exceeded in numerous cases. The samples in this study were analyzed within 2 hours from receipt of material.

The »day-after-day« inaccuracy is highest in the negative control range, where the expected value is 0 particles/mL with deviation up to 20 particles/mL, which influences the high CV. On the contrary, for ranges determined by the values 500 and 1000 particles/mL low CV was obtained (2.8–4.2, i.e. 0.8–4.3). Similar data were obtained in the study prepared by Lamchiagdhase and al. (10), where the CV in the »day-after-day« inaccuracy testing in the
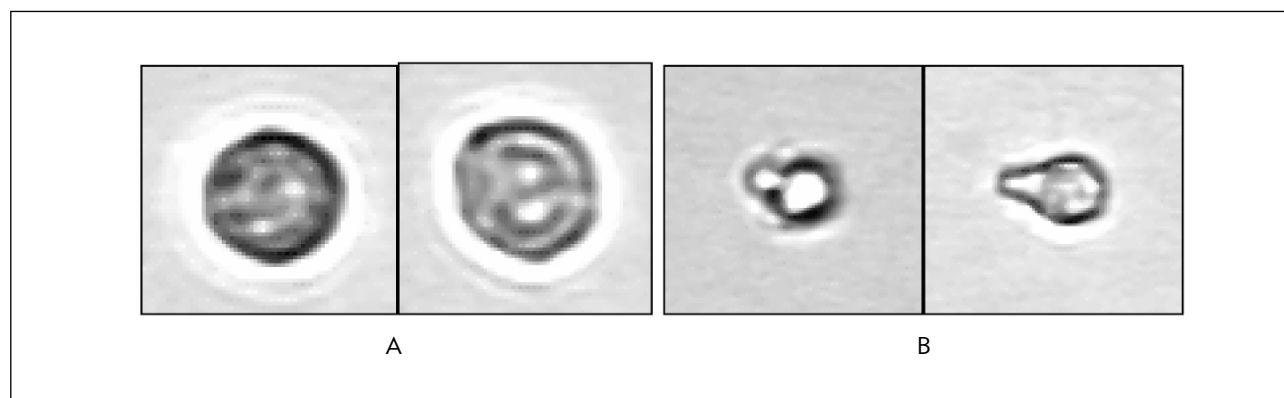
Figure 3 Illustration of leukocytes (A) and dysmorphic erythrocytes (B) in urine – IQ200 microscope module.

case of negative control was 61.6% (range 1–4 particles/mL), i.e. in the case of positive control 6.4% (range 855–117 particles/mL). Also in the study prepared by Wah et al. (11) CV value was »day-after-day« from 3–45%, i.e. within the series 3.3–19%.

For all the analyzed concentration ranges, the values were 97.2%–98.5%, which practically confirms the linearity specified by the manufacturer – up to 1000 particles/mL. In the study prepared by Sikirica et al. (6), these values were ranging between 98–100%. This points to the fact that all samples with the number of particles above 1000/mL should be diluted in order to avoid overlapping of this large number of particles within one frame and consequently, incorrect categorizing. Furthermore, dilution of samples for microscopic analysis should also be necessary in cases when high concentration of certain substances (ex. glucose, proteins, etc.), is determined by chemical analysis

It has been observed that for erythrocytes and leukocytes analyzed by automated microscopy more findings are detected with the presence of these two sediment elements, as well as a higher number of pathological findings, especially for leukocytes (*Figure 3A*). This points to the advantages of automated microscopy in comparison to manual microscopy, probably due to the fact that no previous centrifuging is required, which causes disintegration of these elements. The majority of pathological leukocyte findings determined by automated microscopy, but not by manual microscopy, were boundary values (6–10/mL). Analogously with the other users of this system (6, 11), it was also noticed in this research that the analyzer categorizes the calcium oxalate crystals as erythrocytes, which was not the case with fungi. It can also happen, although rarely, that the urine acid crystals are categorized as leukocytes by the analyzer. Therefore, it is necessary to verify every finding by controlling each category separately, when there is a possibility to transfer the inaccurately categorized elements into the appropriate category.

Until recently, automated urine analyzers did not have the possibility to categorize dysmorphic erythrocytes. However, through constant improvement of technical characteristics by manufacturers, this possibility has also been introduced, registering the dysmorphic erythrocytes (*Figure 3B*) as a percentage of the total number of erythrocytes.

The analyzer recognizes cylinders as hyalines and unclassified cylinders. Therefore, it is necessary to control the category of unclassified cylinders, but also the category of artifacts, and then state in comments (which the operator can introduce in the finding by keyboard) the type of distinguished cylinders. Although hyaline casts are translucent, and some might have been missed by the reader, leading to falsely low cast counts (11), no advances were noticed for IQ200 in cylinders' readout in comparison to manual microscopy. Also, no better readout of crystals was noticed for IQ200 in comparison to manual microscopy. The reason is probably in the fact that IQ200 assorts crystals in some other categories, when it is necessary to perform checkup and transfer of crystals into the right category.

Fungi are relatively well recognized by the analyzer; while among bacteria the recognition of cocci type is more difficult, which is also generally the identification problem with manual microscopy.

The results obtained by a comparative analysis of Multistix sticks and AX-4280 mostly manifested satisfactory compatibility. Testing pH value provides remarkable correlation (r=0.63, p<0.001), as well as testing specific gravity, but to a somewhat lower degree (r=0.32, p<0.01). Values obtained by chemical analysis with AX-4280 can be represented quantitatively or semi-quantitatively. The semi-quantitative method is apparently more acceptable because of the existence of some cases with discrepancies in numerical values in microscopic and chemical analyses (usually in the category of leukocytes and erythrocytes), which can confuse the interpreter of findings. Moreover, in some cases the chemical

analyses provide negative findings of erythrocytes and leukocytes, but the microscope analyses provide expressive positive findings. Various parameters can cause faulty negative findings in chemical analyses, but careful checkup of microscope findings should also be performed due to the possibility of inaccurate categorization of certain elements by the analyzer. After exclusion of these elements, chemical and microscopic analysis provides compatible findings.

Although automation has already been widely used in laboratory diagnostics, in urine analysis it still does not have such an important role as in hematological diagnostics. Manual microscopy and utilization of various sticks for chemical analysis are still common in almost all laboratories. Although these are standardized methods, they imply certain imperfections like subjective evaluation by the analyst and the necessity of urine sample preparation, which also causes certain inaccuracies in manual analysis. Utilization of urine samples that do not require any preparation procedure (ex. centrifuging), complete standardization of analytical procedures, thus avoiding subjectivity of the analyst, as well as shorter time required for sample analysis provide great advantage for the automated system in comparison to manual technique in routine operation. However, apart from all the advantages of automation, presence of an analyst is still necessary for controlling these systems for timely correction of contingent errors of the analyzer, as well as for checkup of certain analyses by standardized, routine methods.

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