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Original Contribution

CURRENT GENERAL AND MICROSCOPIC URINE ANALYSIS IN THE ROUTINE (CLINICAL LABORATORY) PRACTICE IN BULGARIA

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ABSTRACT

Urine analysis is used as diagnostic method in many patient related cases: 1. to confirm a diagnosis; 2. to monitor the applied treatment; 3. for screening the asymptomatic patients. In this case, automatically analyzed test strips are used and in some cases, test strips with more sensitive reaction fields are used.

Task: To present the authors experience in the application of current automated general and microscopic urine analyzes in laboratory practice.

The clinical laboratories of Military medical academy, the University hospital and the district hospitals of five towns have been using automated urinalysis systems for more than two years. Thousands of routine samples were analyzed, either general samples or with a microscopic analysis. An increase in the production rate was observed. The sediment elements are classified in 12 groups by the automated microscopic analyzer. In more samples casts, round shaped epithelial cells (reno tubular and urothel cells), mucus and crystals are identified. The emphasis was on the ability to use routine test strips and strips with reaction fields for microalbuminuria, creatinine and albumin/creatinine (ACR). ACR is an index used for screening purposes and for proteinuria control. It can be confirmed by quantitative determinations. Reliable results are obtained from a correct sample, submitted by a good informed patient within the time limit, in a single use container. It is essential for the laboratory that good communication with the clinic is in existence.

Conclusions: The automation of routine urine analysis contributes to the technician's workload rationalization, increases productivity and the requirements for the technicians become higher. When using automated analysis the technician becomes an expert – verifies, controls and confirms the automatically detected problem findings. The technician can select and examine samples from the computer screen, but also microscopically. Working with automated analyzer is mastered easily. These analyzers are recommended for application in hospital and outpatient laboratories.

Key Words: urine analysis, sediment, renal diseases, microscopic analysis, automated analyzer

INTRODUCTION

Examining (observation) of the urine to diagnose and monitor the patient's condition was performed in medieval times. This probably is The first "laboratory" test. **Figure 1** is a picture, drawn in 1580, that illustrates doctors, assessing a urine sample, brought by the patient in a bladder shaped container called matula. Frederik Deckers describes that the urine, containing protein, precipitates when acidified and heated. This first real publication of in vitro test is dated 1694.(1)

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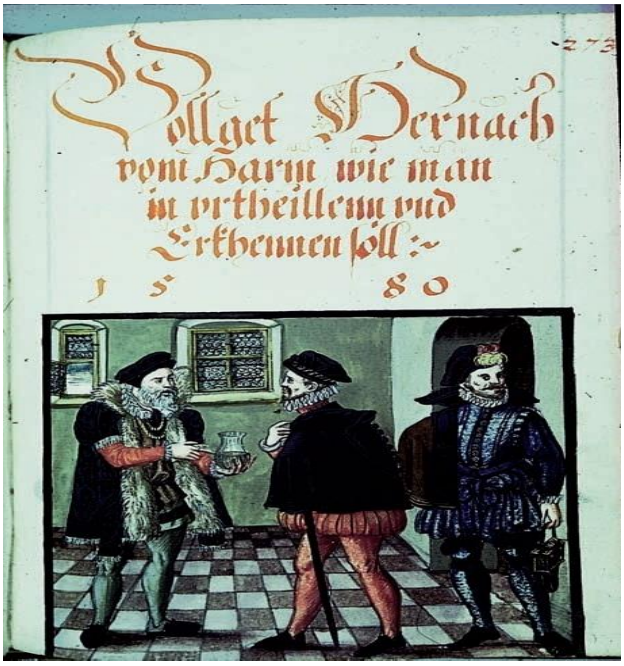

Figure 1. Doctors "discuss" patient's urine sample brought in a special container – matula
The drawing is dated 1580

Table 1 describes some steps of the development of laboratory analysis and techniques used in urine diagnostics

Table 1. *Chronology of main laboratory analysis and techniques (7 with modifications)*

Time interval	Analysis
Medieval times	Inspection – general characteristics; taste (?)
XVI century	Inspection – general characteristics
The end of VII century (1694)	In vitro confirmation of protein in the urine Fr. Deckers
XVIII and XIX century	Urine microscopic elements are described
XX century – the first half	Application of quantitative microscopic analysis – Th. Addis
XX century – the second half	Analytics – routine application of dry chemistry in urine
20-th century – last two decades	Technical devices – stripreaders (reflexion photometry)
	Technical devices for "sediment microscopy" – microscopic and flowcytometric principles

The automation of clinical laboratory urine analysis follows the automation of diagnostic haematology and clinical chemistry. According to the 2011 annual reviews of the author`s laboratories urinalysis take more than 11% of the general workload of the outpatient labs. (2)
THE GOAL of this work is to present the authors` experience in the application of current automated methods for general and microscopic urinalysis in the routine laboratory practice

TASKS
To present:
- the workstation H800/FUS100
- reference values of automated microscopic analysis in urine samples
- achieving better quality by teamwork in the hospitals

MATERIALS AND METHODS
Three workstations H800/FUS100, one combination of H300/FUS100 and three single automated urine dry chemistry analyzers H800 are being used in the authors' laboratories. Thousands of ambulant and hospital patient samples have been analysed. We have tested the productivity of the workstation and the imprecision of the automatic counting.

RESULTS AND DISCUSSION

The machines with semi- and fully automated reading of multiparameter test strips are closed systems - strips and readers are from the same manufacturer. (3, 4) Multiparameter strips for common urine anysis, but also - with reaction fields for microalbumin (MA) and creatinine (Cr) are on sale. (5, 6) Last two parameters are the basis for calculation of albumine creatinine ratio (ACR) by every routine urine test. One important advantage of the strip readers is the objectie reading of chemical reactions at a defined time intervals. The results obtained with DIRUI H 11 test strips and Autionstiks - Arkray – Japan have been compared. It has been proven that more than 93% of the results are concordant. We have estimated that the maximum production rate of H500. It is ≈ 100 samples/hour (when performed by well-trained and skilful technician). For H800 the estimate is 200 samples per hour. **Table 2** presents the productivity of the workstation H800/FUS100, tested chronometrically.

Table 2. *Working times of series with different lengths analyzed on H800/FUS100*

Number of samples	Time (minutes)	Average time per sample (min)
1	2,5	2,5
10	15	1,5
232	232	1,0

In order to obtain reliable results urine samples must be mixed well before loading them on the workstation. The automated microscopic Flow Urinary System – FUS 100 receives the sample tubes from H800, mixes the current sample and aspirates the necessary volume prior to running it through the flow cell in front of the microscopic objective. The digital camera takes 820 photos (frames) per sample. This equals to 820 microscopic fields being analyzed **(Figure 2)**. (5, 7)

Figure 2. Picture of a microscopic viewing field

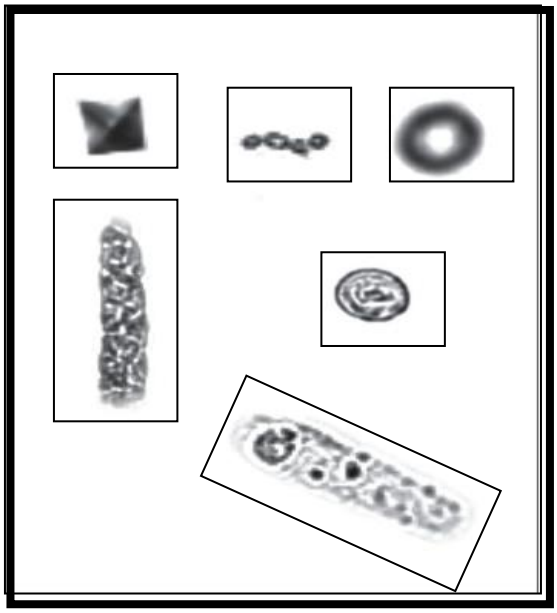

Figure 3. The software identifies separate elements in the frame prior to classify them

Blinking illumination makes the photographed objects “immobile”. The software detects all elements in the frames **(Figure 3)**, compares them to a database and classifies the separate elements in 12 main groups = the elements of urine sediment. The operator verifies all the results. He/she has to compare the dry chemistry results and the microscopic findings, shown

together on the computer screen; to correct and/or verify the results; can select samples for "manual" microscopic analysis. In the end of the analysis, the workstation generates a combined result – dry chemistry- and microscopic results in the form of integrated findings and prints or sends them to LIS. The automated microscopic analysis detects more frequent hyaline- and pathological casts, round epithelial cells, mucus and some crystals. The machine "reminds" the technician to confirm or deny an automatically detected finding. It is possible to select the output of the microscopic result: numbers per low power field (LPF), # per high power field (HPF), #/µl or - with symbols (-), (-/+), (+), (++), (+++).

The calculated imprecision shows a low CV in the sample with a higher cell count and vice versa, high CVs in such - with lower count – **Tables 3 and 4**.

Table 3. *Serial imprecision – RBC counts with FUS100*

RBC counts			
Range (RBCs/µl)	0	21-25	1333-1493
Mean (RBCs/µl)	0	24.0	1530
SD (RBCs/µl)	0	1.6	52.1
CV (%)	0	6.4	3.4

Table 4. *Serial imprecision – WBC counts with FUS 100*

WBC counts			
Range (WBCs/µl)	0	4 – 6	64 – 80
Mean (WBCs/µl)	0	4.0	71.0
SD (WBCs/µl)	0	0.61	5.2
CV (%)	0	14.1	7.3

The RBC and WBC results, obtained automatically are compared by the linear regression calculation with the manual chamber counts (5). Y = automatic results, x = manual results.

Erythrocytes: Y = 1.150x +4.9 (n=40, min 0, max 134 /µl). r = + 0.936
Leucocytes: Y = 1.237x -3.8 (n= 49, min 0, max 232 /µl). r= + 0.925
We prove extremely highly positive correlation between the two counting ways and suspect that the higher results, obtained by the automatic method are due to their enumeration in a greater sample volume.

Table 5 summarizes the cut-off values for urine sediment elements recommended by some authors. External genital cleaning prior to voiding seems to be an essential factor (8), which is, applicable in hospital patients.

Table 5. *Cut-off values, separating the normal from the pathological values, as published in three separate sources*

	RBC (#/ul)	WBC (#/ul)	Sq. ep. cells(#/ul)	Casts	Autor	
DIRUI - factory set	< 17	< 28	< 28	<1 LPF	(7)	*
Fogazzi G.B.	< 1/HPF	< 1/HPF	Low	< 1 LPF	(8)	
Corresponds ≈ #/µl	< 6	< 6	Low	Low/LPF		**
Sysmex UF1000i ♂	<13.0	<9.2	<5.7	<2.25	(9)	
Sysmex UF1000i ♀	<30.7	<30.7	<45.6	<2.4		***

* No data for external genital cleaning prior to voiding
** Urine voided after genital cleaning with water
*** Ambulant patients, wellness testing without genital cleaning

Teamwork between the laboratory and the clinical staff is very important. Convenient single use urine sample containers are on sale. If freshly collected samples are transferred from the primary containers into hermetically closed and bar coded tubes on site in the wards the analyses are easily performed. This allows small, but adequate for the automated analysis, volumes of urine to be transported, with the lowest possible infectious risk for the medical staff.

CONCLUSIONS

The automation of routine urinalysis:

1. Contributes to the technician's workload rationalization
2. Increases the productivity and the requirements for the technicians become higher
3. The technician becomes an expert – verifies, controls and confirms the automatically findings
4. Allows to apply a LEAN management within the urine laboratory
5. Reduces the risks of mistakes and infections for the laboratory staff
6. Contributes to greater reliability of the results
7. We recommend it for wide application in hospital and outpatient laboratories.

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