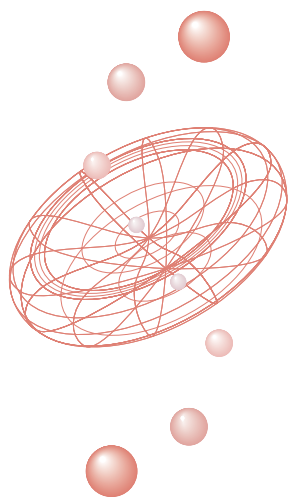


**REVIEW**

**ARTICLE**



# Bringing Urinalysis into the 21<sup>st</sup> Century: From Uroscopy to Automated Flow Cytometry

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## HISTORY

Already before the advent of modern medicine, urine was used for medical diagnosis, pre-dating quite probably even the "father of medicine" in Western culture, Hippocrates. At times - as with many ancient ideas on medicine - this reached heavily into superstition and esoteric practice. Extremes of this development ranged from diagnosis solely based on urine, sometimes without ever seeing the patient, up to and including fortune-telling<sup>1)</sup> (cf. also *Fig. 1*). This reached a climax in the 17<sup>th</sup> century, leading to the publication of the devastating text "*Pisse Prophet*" by Thomas Brian ridiculing this development and bringing uroscopy, as urinalysis was called, under the suspicion of charlatany<sup>1,2)</sup>. However, more scientific microscopic examination of urine can be traced back to the 17<sup>th</sup> century as well and thus constitutes one of the oldest techniques used in the medical laboratory. Initially, the focus was on the description of crystals found in urine. From the beginning of the 19<sup>th</sup> century onward, the technique was practiced more and more systematically; the pre-analytical sample-handling was methodologically described and the use of the technique diversified with the description of dysmorphic erythro-

cytes, squamous epithelial cells and casts. Likewise, the importance of cross-linking the results of the microscopic investigations with chemical investigations was more and more emphasized. Rayer and Vigla of the Hôpital de la Charité in Paris became the front-runners in the development of urinalysis at that time, characterising pyuria and haematuria on the microscopic level. By today, this practice once thought to be charlatany spawned not just modern urinalysis, but also contributed to the birth of endocrinology and nephrology<sup>2)</sup>.

Another "offspring" of microscopy, so to speak, was the field of scientific microbiology. While microorganisms had been postulated as far back as the last century B.C., it was only with van Leeuwenhoek's discovery of "little animals", protists and bacteria, in the first scientific uses of the newly-developed microscope in the late 17<sup>th</sup> century, that their existence could be confirmed. It took another two centuries, until the germ theory was again supported, and finally proven, by Robert Koch, who, dissatisfied with liquid media for some applications also developed the first solid media for culturing bacteria. Thus, there are now two major ways to culture bacteria also for urinalysis: Quantitative cultures and solid dipslides.



**Fig. 1** Historical "urine wheel" for uroscopy

Used to diagnose various diseases based on the colour of the patient's urine. The colour is compared to the flasks pictured and the annotations will specify the state of digestion and lead to the "proper" diagnosis as well as prognosis.

Almanac, England [York?], n.d. [1406-1424], MS 1004/29 Rosenbach Museum and Library, Philadelphia, USA, reproduced with kind permission.

Microscopy and bacterial culture have been critical for analysing urine in the 20<sup>th</sup> century. The former allows to detect the presence of both endogenous cells such as white (WBC) and red (RBC) blood cells and epithelial cells. The latter allows the detection of even relatively low concentrations of bacteria by multiplying them in culture. In addition, test strips have been developed both to give a general idea whether these cells might be present in urine and to assess a number of clinical-chemical parameters in a semi-quantitative fashion. These can only detect gram-negative bacteria and only under specific circumstances. Still, a positive result is a quick and good indication for the presence of an infection of that subset of uropathogenic organisms. All these technologies are at the disposal of the modern physician to be used in the diagnosis of infections in the urinary tract and other sources of changes in urinary composition.

## URINARY TRACT INFECTIONS, THEIR DIAGNOSIS AND TREATMENT

When bacteria intrude into the lower urinary tract, they often cause a urinary tract infection (UTI), a common

diagnosis in clinical practice. Often, the cause is *Escherichia coli* (*E. Coli*), migrating from the colon to the urethra. Due to the shorter urethra, infections beyond the urethra such as cystitis are much more common in women than in men. UTIs cause pain during urination, frequent urge to urinate, while actual voiding of the bladder is hindered. In addition, colic pain occurs in many cases. Left untreated, the infection can spread to the upper urinary tract and potentially cause pyelonephritis. Since this can eventually lead to permanent kidney damage and urosepsis, and not the least because of the extensive pain caused to the patient, early diagnosis is desirable to avoid complications and further pain.

Since the discovery of penicillin by Alexander Fleming in 1928 and the development of other antibiotics, the subsequent treatment of infections has - at least until the rise of multi-drug resistant bacteria - largely been unproblematic. However, the very notion of resistant bacteria, along with the costs of treatment and the burden any drug treatment poses for patient physiology, mandate that an antibiotic treatment is initiated only where really necessary. An international survey of the antimicrobial susceptibility of urinary pathogens - the ECO-SENS project - illustrated that only 60% of uropathogenic strains of *E.coli* are still susceptible to standard antibiotics. The interest in avoiding further resistances should thus be obvious<sup>3,4)</sup>.

For cells of human origin found in urine, such as WBCs, RBCs and epithelial cells, despite the variability inherent to analysis done directly by human beings, results have been satisfactory. However, this process is time consuming and greatly dependent on the individual skills and experience of the examiner. In addition, statistical considerations greatly limit the optimally achievable result by direct microscopy by humans on the basis of number of particles counted. Since microscopy is usually done on urine sediment, it also requires centrifugation of the urine. However, the different cell types do not sediment uniformly. Hannemann-Pohl and Kampf found for example that only 73% of leukocytes actually are found in the sediment - with a CV of 18%<sup>5)</sup>.

At the same time, bacteria culture is even more time consuming, requiring one, and sometimes two full days until results can be observed. Depending on the type of bacteria, the culturing conditions might prevent bacteria from growing altogether.

This often leads to treatment initiation before the actual results of the diagnostic tests are available. The very notion of resistant bacteria, along with the burden any drug treatment poses for the patient and the cumulative costs of such treatments are reasons for concern about this practice. This is all the more the case if the patient is already hospitalised and a potential infection would be nosocomial. A 2005 study of the costs of antibiotics treatment for nosocomial infection in a Turkish university hospital estimated the costs of UTIs to a mean amount of US\$ 52.37 per day. However, the range of US\$ 8.56-226.68 illustrates that this mean relates to relatively uncomplicated cases and drug-resistant strains can cause

costs more than four times the mean. Overall, UTI is not only the second most frequent but as a consequence also the second most expensive in total treatment costs of nosocomial infections in the study after pneumonia <sup>6)</sup>. The cost factor introduced by drug-resistant bacteria in UTI thus is obvious. In some countries, resistance frequencies in individual strains can even be much higher than cited in the ECO·SENS study. A review published in 2005 cites studies e.g. from Spain reporting sometimes a quarter and in some cases even half of the strains resistant against the frequently used cotrimoxazol and already one third resistant against second-generation cephalosporins <sup>7)</sup>. This is caused not the least by indiscriminate prescription of antibiotics without identifying the pathogenic organism and its susceptibilities. Thus, it is imperative to curb this trend and prevent further misapplication of antimicrobial therapy from causing further resistances.

With the advent of automated analytical systems, solutions have been developed to automate the counting of particles in urine. Three goals are pursued in this development:

- to reduce the timeframe in which results can be expected (turnaround time) and thus avoid treatment

in cases in which it is not needed and

- to free the highly qualified personnel for cases in which their expertise is actually needed instead of occupying them with readily distinguishable negative cases.
- to allow the generation of reproducible results with standardised procedures.

This can be done directly using the urine sample, without centrifugation, thus avoiding problems of varying sedimentation rates.

Technical solutions for this problem can be based on flow cytometry. To this end, cells in urine can be stained with fluorescent dyes for membranes and/or DNA. The combined analysis of scattered light and fluorescence allows the identification of particles as leukocytes or bacteria or even yeast-like cells. Analogously to automation in haematology, this allows a drastic reduction of turnaround time. The sample throughput of such analytical systems can reach up to 100 /hour. Sysmex has long provided solutions in this area and with its experience in fluorescence flow cytometry brought them into the new millennium (**Fig. 2**).



**Fig. 2** Bringing urinalysis into the 21<sup>st</sup> century with Sysmex urinalysis solutions

Top row: the 1990s: left: UF-100, introduced in 1995; right: UF-50, introduced in 1997; Middle: the new millennium: left: BACSYS-40i, introduced in 2002; right: UF-100i, introduced in 2004; Bottom: the new UF-1000i, introduced in 2006

Especially the newer techniques offer a good concordance with clinical findings and traditional methods. Discrepancies in bacterial counts between automated counting and culture occur where bacteria can be found in urine that are dead or growth-impaired. These are found in automated counting - regardless of the specific method used - but not in culture. Thus, Gässler found some samples from clearly or potentially infected patients based on clinical observations to be definite or potential cases of UTI in automated counting with the Automated Bacteria Analyser BACSYS-40i (Sysmex Corporation, Kobe, Japan) that were negative in culture <sup>8)</sup>. Already with the fully Automated Urine Cell Analyser, UF-100 (Sysmex Corporation, Kobe, Japan, hereinafter called "UF-100"), Kouri expected a time-saving effect

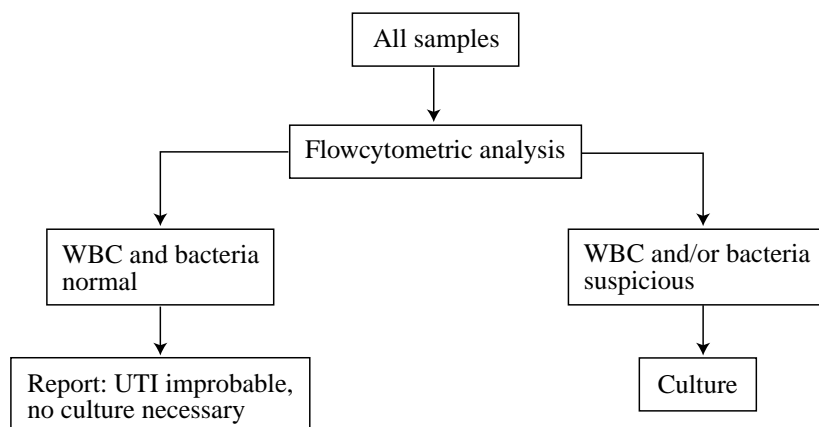
through avoiding unnecessary microscopy of 6h/work day - already taking into account the time necessary for the automated analysis, just 72 seconds per sample for the UF-100 <sup>9)</sup>. This prevents highly qualified personnel from being tied up with routine tasks and allows full focus on those problem cases actually requiring their level of competence and attention for correct analysis. The time-saving effect also frees resources for additional samples, thus increasing the daily throughput of the laboratory.

Meta-studies demonstrate the significant decrease in samples requiring microscopy even in laboratories already analysing only a minor percentage of samples via microscopy before introduction of the automated system <sup>10)</sup>.

**Table 1** Reduction of samples for microscopy by introduction of an automated urinalysis solution in several studies

Author	Fractions of samples examined through microscopy before introduction of automated urinalysis (%)	Fractions of samples examined through microscopy after introduction of automated urinalysis (%)
Hannemann-Pohl	100	10-20
Delanghe	80	15
Kouri	60-70	20-35
Fenili	100	14
Lun	36	6

*This Table adapted from Delanghe et al. Table 2 <sup>10)</sup>*



**Fig. 3** Suggested algorithm for the processing of urine samples in the diagnosis of UTI

Actual cut-off values vary between studies, ranging from 20-45 for WBCs, and from 1800-3000 for bacteria. They should be adapted to the actual setting of patient population and sample material as well as the analytical system used and the specificity and sensitivity desired. Upon detection of an infection, the culture can then be used to identify the organism and its susceptibilities to antimicrobial agents.

**Table 2** Comparison of the analytical performances of quantitative evaluation on CLED agar and the UF-100 in the diagnosis of UTI

Method	Sensitivity	Specificity	PPV	NPV	Correct clinical classification
CLED Agar	0.89	0.98	0.93	0.96	0.95
UF-100	0.94	0.93	0.83	0.98	0.93

This Table adapted from Manoni et al. Table 2<sup>13)</sup>

Analogously, other studies show a reduction of bacteria cultures of up to 75%, depending on what ratio of false-negative results is acceptable<sup>11,12)</sup>. Thus, the average sample turnaround time is drastically reduced. Assuming a turnaround time of 72 seconds per sample, 30 samples can be analysed in 36 minutes. For negative samples, this already concludes the sample analysis. If this is the case for example for 50% of the samples, the statistical mean turnaround time is - aside from the system analysis time - also reduced by half.

As a consequence, a workflow as **Fig. 3** can help to avoid the unnecessary use of time-consuming analytical techniques.

In routine cases of initial presentation with symptoms of UTI, the analyser result alone may be sufficient for diagnosis. In recurring disease, pregnant women, hospitalised patients and other complex cases, a positive analyser result should in any case lead to bacteria culture for identification of the pathogenic organism and screening for its susceptibilities.

A field evaluation of the UF-100 by Manoni illustrated how reliably the automated analyser can classify actual patient samples, demonstrating a performance similar to that of CLED (Cystine-Lactose-Electrolyte Deficient) agar cultures and an excellent negative predictive value surpassing the bacterial culture<sup>13)</sup> (**Table 2**).

## CONCLUSIONS

Urinalysis, one of the oldest medical techniques, has arrived in the 21<sup>st</sup> century with new analysers such as the fully Automated Urine Particle Analyser UF-1000i (Sysmex Corporation, Kobe, Japan). Time-consuming methods dependent on significant expertise can be saved for those cases in which such expertise is actually needed and negative cases can be quickly identified. This decreases not only costs in terms of actual money and time but also the risk of unnecessarily generating further resistances against antibiotics. It should also improve the accuracy of those cases which can now benefit from the full and focused attention of highly trained personnel who were previously dealing mostly with negative cases. While "dipstick" test strip analysers for UTI can apparently offer similarly quick results, they detect only a certain subset of infections under specific conditions.

Enterococcus, for example, as a gram-positive microorganism, will not be detected by test strips while in some studies being cited as the second most common uropathogenic organism<sup>7)</sup>. In addition, - especially when used in a point-of-care environment - they tempt to skip the antibiotics susceptibility screening, thus leading either to an unnecessarily expensive treatment with broad-spectrum antibiotics or the useless treatment with agents the pathogenic microorganisms are resistant against. The use of automated systems allows reproducible and independently comparable assessment of the WBC and bacteria concentration in urine, a reliable screening procedure for UTI and consequentially an important improvement of the laboratory workflow. While legal provisions from Jerusalem of 1090 included a provision for the public beating of physicians who diagnosed disease without looking at the urine, in the 21<sup>st</sup> century, the physician can rely on the proven technology of a Sysmex UF analyser to perform this task for him.

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