Technology assessment of automated urine particle flow cytometry

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Abstract. Recently, urine particle flow cytometry (UFC) has become available as an alternative method in urinalysis. A complete analysis can be carried out with only 800 microliter of sample. The instrument is characterised by a high sensitivity and linearity. UFC is more precise than sediment microscopy. Correlation with counting chamber analysis is excellent. The better analytical performance is the major advantage of the technique. Fast availability of the data is another advantage of the technique. From an economical point of view, introduction of UFC reduces labour costs. Optimal results are obtained when expert systems are used for interpreting the data. The superior analytical quality of UFC should however be accompanied by a growing attention for the pre-analytical care. The largest contribution to the total error undoubtedly occurs during the pre-analytical phase. Combining the diagnostic information of UFC with the information provided by urinary test strips and the dosage of specific urinary proteins will provide the clinician with extra information.

Introduction

The examination of the urine remains one of the most commonly performed tests in clinical laboratory practice. For over a century, standard microscopy analysis of urine has been considered as a golden standard. However, critical evaluation of sediment analysis has revealed a number of significant flaws, particularly in relation to centrifugation, the transfer error from the centrifugation tube to the microscope and the relatively high counting errors due to the relatively low number of elements counted[1]. Recently, urine particle flow cytometry (UFC) has become available as an interesting alternative method in urinalysis[1-6]. The present article reviews the analytical performance of UFC and the utility of this novel analytical technique in the clinical routine laboratory.

Description

The urinary flow cytometer can identify red blood cells (RBC), white blood cells (WBC), squamous epithelial cells, small round cells (this latter category includes both renal tubular cells and transitional epithelial cells), hyaline casts, pathological (inclusional) casts, bacteria, yeast-like cells, spermatozoa and crystals. The exact nature of the urinary crystals cannot be characterised by the instrument. Unidentified particles (e.g. Trichomonas parasites) are referred to as “other cells”. Results are displayed as scattergrams and histograms and numerical values are reported (as particles per microliter)^{2}. Additionally the system provides a flag for RBC size distribution. A complete analysis can be carried out with only 800 microliter of sample. The sample is diluted by the instrument. The dilution buffer stabilises the osmotic pressure of urine within a defined range thus enabling the impedance measurement. The urine sample is heated to 37 °C to dissolve amorphous urates, and, after staining with the dyes carbocyanine and phenanthridine, enters the flow cell. Carbocyanine stains the cell membrane and phenanthridine stains nucleic acids. The particles are hydrodynamically focused; an argon laser (wavelength 488 nm) encounters these particles and particle-specific forward light scatter and fluorescence signals are emitted. Based on the physical characteristics of forward scatter, forward scatter pulse width, fluorescence, fluorescence pulse width and impedance, the urine particles are classified. Conductivity is simultaneously measured as an indicator of the patient’s hydration status. Conductivity can help in the interpretation, especially when the result is showing borderline normal results or slightly outside the reference range. The UF-100 instrument (Sysmex) can handle 100 urine samples per hour. The instrument is factory calibrated but this is verified at installation by total WBC and RBC count procedures using latex particles supplied by the manufacturer.

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Technical performance

The instrument is characterised by a high sensitivity: lowest limit for RBC detection was 2.3 10^6/L, for WBC 3.2 10^6/L, for epithelial cells 0.3 10^6/L. Linearity has been established up to 4.5 10^9 WBC/L, 3.5 10^{10} RBC/L and 2.5 10^{11} total particles/L[2,4]. Within-day precision and between-day precision for the various particle counts showed the UFC to be consistently and significantly less imprecise than for sediment microscopy. Sample carry-over is negligible due to the automatically performed rinsing steps. Correlation with counting chamber analysis[1,5] of native urine or test strip analysis[3] is excellent. The addition of preservative agents such as formaldehyde and bore acid does not interfere with instrument performance.

Localisation of sites of hematuria

In a number of cases, the location of hematuria can be suggested by the size and shape of the erythrocyte. However, relatively few clinical studies have validated the use of UFC for differentiation between eumorphic from dysmorphic erythrocytes.

Application strategies

Today there are various strategies for urine examination[2]. UFC is well suited for primary screening. UFC produces more precise and accurate counts than reflectance chemistry used for automated test strip reading. There is still a need for microscopy review in a small minority of cases. In many microbiology labs, UFC has been implemented as a first screening for diagnosis and monitoring of urinary tract infections (UTI). Results generally correlate well with those of bacterial cultures[2,4-7]. The UTI screening approach quickly provides information to the clinician and greatly reduces the number of urine cultures to be carried out by the clinical lab. However, attention should be paid to the existence of different algorithms for counting bacteria by flow cytometry, which limits the transferability of test results[7].

Other clinical applications of the technique

Next to the intended use in urine, urine flow cytometers can also successfully be used for the analysis of peritoneal dialysis fluid in patients on continuous ambulatory peritoneal dialysis (detection of peritonitis)[8]. Even particle analysis of cerebrospinal fluid, saliva or semen can be carried out using the same equipment[9-11].

Clinical utility of UFC

The better analytical performance is the major advantage of the technique. As no centrifugation step is needed during the analysis, less cellular material is destroyed during the sample manipulation which results in a more accurate counting of the brittle elements (e.g. casts). Trichomonas parasites are not recognised by the instrument, however in case of Trichomonas infection flaggings for other fields usually draw the operator's attention. Although the instrument is not capable to define the exact nature of urinary crystals, the clinical utility of crystal differentiation in the urinary sediment is generally low. The lesser required sample volume is a great advantage in pediatrics where obtaining the volume required for standardised microscopy (10 ml) is not always possible. The fast availability of the data is another advantage of the technique. Experience in interpretation of the scatter grams is helpful in further reducing errors. From an economical point of view, the introduction of UFC reduces labour costs in the middle – sized and bigger clinical laboratories.

Expert systems

Expert systems have been developed for checking urinalysis results. The expert system rules systematically check the data of the instrument and compare results of UFC with those of urinary test strips (e.g. RBC count with hemoglobin concentration, WBC count with leukocyte esterase). Optimal results can be obtained when quantitative reading of the test strip (reflectance data) can be carried out. The application of expert systems has the main advantage that the error rate is greatly reduced and the review rate is kept to a minimum. Expert systems are also capable of detecting samples of poor pre-analytical quality (e.g. by analysing the WBC: bacteria ratio)[3].

Despite the existence of these expert systems, visual microscopy will still be required in a small number of cases. These include differentiation of casts, renal tubular epithelial cells, dystrophic RBC, certain micro-organisms (fungi, Trichomonas vaginalis and other parasites). Also the occurrence of rare pathological crystals (e.g. cystine, tyrosine and 2,8 dihydroxyadenine) in the sediment still needs visual microscopy. In the near future, commercial availability of test strip readers that also provide the reflectance readings will allow a further refinement of the expert system review process.

Impact on workflow

Various studies have confirmed large reductions in the need for sediment microscopy compared to the previous workflow. Microscopy rate drops to 15-50% of the rate before introduction of UFC[2].

Future developments in urinalysis

UFC is proving to be an interesting and informative technology which has produced a revolution in urine particle analysis. The analytical precision of UFC is superior to that of visual methods due to the fully automated and therefore standardised process. The superior analytical quality of UFC should however be accompanied by a growing attention for the pre-analytical care of the urine sample. The largest contribution to the total error undoubtedly occurs during the pre-analytical phase. Combining the diagnostic information of UFC with the information provided by (quantitative) urinary test strips[3] and the dosage of specific urinary proteins (such as micro albumin, alpha 1 microglobulin, alpha 2 macroglobulin)[12] will provide the clinician with extra information. Quantitative test strip analysis and specific protein assays offer the additional advantage having CV values which are comparable to the ones for UFC. Further clinical studies will be needed to explore the possibilities of UFC for the diagnosis of nephrological and urological diseases.

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