AJKD
Narrative Review

Advances in Urine Microscopy

Gavin J. Becker, MD,[1] Giuseppe Garigali, MD,[2] and Giovanni B. Fogazzi, MD[2]

Urine microscopy is an important tool for the diagnosis and management of several conditions affecting the kidneys and urinary tract. In this review, we describe the automated instruments, based either on flow cytometry or digitized microscopy, that are currently in use in large clinical laboratories. These tools allow the examination of large numbers of samples in short periods. We also discuss manual urinary microscopy commonly performed by nephrologists, which we encourage. After discussing the advantages of phase contrast microscopy over bright field microscopy, we describe the advancements of urine microscopy in various clinical conditions. These include persistent isolated microscopic hematuria (which can be classified as glomerular or nonglomerular on the basis of urinary erythrocyte morphology), drug- and toxin-related crystalluria (which can be a clue for the diagnosis of acute kidney injury associated with intrarenal crystal precipitation), and some inherited conditions (eg, adenine phosphoribosyltransferase deficiency, which is associated with 2,8-dihydroxyadenine crystalluria, and Fabry disease, which is characterized by unique urinary lamellated fatty particles). Finally, we describe the utility of identifying "decoy cells" and atypical malignant cells, which can be easily done with phase contrast microscopy in unfixed samples.
Am J Kidney Dis. 67(6):954-964.

INDEX WORDS: Urine microscopy; urinalysis; automation; urine sediment; hematuria; red blood cells (RBCs); acute kidney injury (AKI); crystalluria; cytopathology; phase-contrast; bright-field; polarized light; diagnosis; kidney disease; nephrologist; review.

Gavin J. Becker, MD, was an International Distinguished Medal recipient at the 2015 National Kidney Foundation Spring Clinical Meetings. The International Distinguished Medals are awarded to honor individuals who have made significant contributions to the field of kidney disease and furthered the goals of the National Kidney Foundation.

Microscopic examination of urine is a joint responsibility of nephrologists and pathologists. The former, if sufficiently trained and equipped, are able to obtain important diagnostic information about the individual patients under their care. However, increasingly, a lack of appropriate resources, including time, equipment, biologically safe environments, and, in the United States, legislative barriers,[1] are making this procedure more difficult. Further, urinary microscopy by nephrologists is gradually disappearing in many practices. Pathology departments and practices generally handle large numbers of urine samples on a daily basis and are often forced to delegate microscopic examination of urine to technicians who may not receive fresh appropriately collected specimens or have the time, expertise, and equipment to derive the most information from each individual specimen. The purpose of this review is to bring to the attention of both groups recent advances in urine microscopy in the hope that this will rekindle interest in solving these intellectual and logistic problems.

AUTOMATED URINARY MICROSCOPY

The challenge of counting the particles in large numbers of body fluid specimens has long been an issue for hematology. Wallace Coulter (1913-1998), an electrical engineer who was stimulated by the need to follow up the large numbers of people affected by nuclear radiation in Japan after World War II, developed a method for counting particles in electrolyte solutions. This technique, known as the Coulter principle, was implemented in 1954 as the Coulter Counter Model A, which enabled rapid and accurate enumeration of cells in blood. Since then, machines based on the Coulter principle have become mainstream equipment in all diagnostic hematology laboratories, confirmed by microscopic examination of blood smears by expert pathologists in selected cases. This has expanded to the use of laser-based flow cytometry to better characterize cell populations. Among other issues, the Coulter principle proved difficult to apply to urinalysis because there is a large variety of crystals, cell debris, and other particles in urine. Not until the late 1990s, nearly 50

From the [1]Department of Nephrology, The Royal Melbourne Hospital and University of Melbourne, Melbourne, Australia; and [2]Clinical and Research Laboratory on Urinary Sediment, UO di Nefrologia e Dialisi, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy.

Received June 11, 2015. Accepted in revised form November 3, 2015. Originally published online January 22, 2016.

Address correspondence to Gavin J. Becker, MD, The Royal Melbourne Hospital, C/O Post Office, The Royal Melbourne Hospital, Grattan St, Parkville, Victoria, Australia 3050. E-mail: gavin.becker@mh.org.au

0272-6386
http://dx.doi.org/10.1053/j.ajkd.2015.11.011

years after Coulter introduced his method, were machines for identifying and counting particles in urine developed. As occurred in hematology, a strategy of combining machines to survey large numbers of samples with expert microscopy of select individual samples has become a practical approach for hospital or centralized laboratory urinalysis. Individual clinicians should become experts in interpreting the raw numbers and be able to check microscopic appearances in specific cases.

There are 2 types of machines for counting urine particles, using either flow cytometry with fluorescent dyes as in modern hemato-oncology or software analysis of digitized microscopic images. The relative advantages and disadvantages of these 2 approaches are summarized in Table 1. Laser-based flow cytometry has been extensively studied and shown to be particularly useful in screening for pyuria, bacteriuria, and hematuria, reducing the need for microscopy or culture.[2-9] However, this produces scattergrams rather than images, and microscopy is still required to differentiate similar particles such as dysmorphic and isomorphic red blood cells (RBCs), types of epithelial cells, and various crystals. It reduces laboratory work because further microscopy or urine culture can be avoided if the number of cells or bacteria is below thresholds set by the operator. Because this is not strictly microscopy, we do not discuss it further in this review.

A more relevant approach to urinary microscopy uses computerized analysis of digitized monochrome images of urinary particles. Two types of instruments supplying digitized images are on the market today. One is based on automated intelligent microscopy (iQ200 by Beckman),[10] whereas the other uses cuvette-based microscopy (UriSed by 77 Elektronika and sediMAX by A. Menarini Diagnostics).[11] The 2 instrument types differ in that the iQ200 shows the particles identified in samples by categories (erythrocytes, leukocytes, etc), whereas UriSed and sediMAX show the particles within whole fields of view, similar to microscopic fields seen with manual microscopy (Fig 1).

One advantage of digitized instruments is that the actual images can be reviewed by the pathologist or nephrologist to inform their opinion regarding patterns. Hence, they are useful for both large sample numbers and close attention to select individual patient samples.[10,11] Because the images are stored in a computer, they can be sent to other sites, including clinics, for review.

In addition to the ability to handle large numbers, automation has other advantages. For example, it eliminates the need for centrifugation, standardizes microscope measuring parameters, and can interface with computerized clinical information systems for transmission, storage, and data analysis, thus avoiding transcription errors. In the more complex systems, urinary biochemical data can be combined with the microscopy analysis to deliver a complete urinalysis report. Furthermore, these technologies, with appropriate modifications, could lead to urine analysis using special microscopic techniques valuable in manual urine microscopy, such as phase contrast, polarized light, and counterstaining for fluorescent microscopy. Larger urine volumes could be surveyed for detecting particles in very low concentration.

These urine microscopy instruments need only small urine volumes (2-3 mL), and fully automated models can handle more than 100 samples per hour. With different manufacturers, technologies, and needs of various users, these instruments come in several models from small manually loaded to fully automatic, with added options such as urinary chemical analysis.

Comparisons with manual urinary microscopy performed by experts and between the various devices show good agreement on such important matters as identification of RBCs, white blood cells, bacteria, and squamous epithelial cells. However, at this stage,

Table 1. Comparison of Urinalysis by Automated Flow Cytometry and Digitized Microscopy

	Flow Cytometry	Digitized Microscopy
Images produced	Scattergrams; no particle images	Black and white particle images, shown either by category or within whole field view (see text)
Particles identified and counted	RBC, WBC, EC; hyaline casts; common crystals; bacteria	RBC, WBC, EC; hyaline casts; common crystals; bacteria
Advantages	Minimum expertise	Screened images that can be reviewed by the operator
Minimum sample volume	4 mL	2-3 mL
Samples per h	100	65-100
Manufacturers	1 FDA approved	At least 3, not all FDA approved
Models	Limited range	Wide range
Approximate cost	125,000 USD	60,000+ USD

Abbreviations: EC, epithelial cell; FDA, US Food and Drug Administration; RBC, red blood cell; WBC, white blood cell; USD, US dollars.