Enhanced vs. Automated Urinalysis for Screening of Urinary Tract Infections in Children in the Emergency Department

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1. ABSTRACT

**Background:** Urinary tract infections (UTI) are the most common serious bacterial infection in febrile infants. Urinalysis (UA) is a screening test for preliminary diagnosis of UTI. Urinalysis can be performed manually or using automated techniques. We sought to compare manual versus automated urinalysis for urine specimens obtained via catheterization in the pediatric emergency department (ED).

**Design:** In this prospective study, we processed catheterized urine samples from infants with suspected UTI by both the manual method (enhanced UA) and the automated method. We defined a positive enhanced UA as $\geq 10$ white blood cells (WBCs) per cubic millimeter and presence of any bacteria per 10 oil immersion fields on a Gram-stained smear. We defined a positive automated urinalysis as $\geq 2$ WBCs per high-powered field and presence of any bacteria using the IRIS iQ200 ELITE. We defined a positive urine culture as growth of $\geq 50,000$ colony-forming units per milliliter of a single uropathogen. We analyzed data using SPSS software.

**Results:** A total of 703 specimens were analyzed. Prevalence of UTI was 7%. For pyuria, the sensitivity and positive predictive value (PPV) of the enhanced UA in predicting positive urine culture were 83.6% and 52.5%; corresponding values for the automated UA were 79.5% and 37.5%. For bacteriuria, the sensitivity and PPV of a Gram-stained smear (enhanced UA) were 83.6% and 59.4%; corresponding values for the automated UA were 73.4%, and 26.2%. Using criteria of both pyuria and bacteriuria for the enhanced UA resulted in a sensitivity of 77.5% and a PPV of 84.4%; corresponding values for the automated UA were 63.2% and 51.6%. Combining automated pyuria ($\geq 2$ WBC/hpf) with a Gram-stained smear resulted in a sensitivity of 75.5% and a PPV of 84%.
Conclusion: Automated urinalysis is comparable to manual urinalysis for detection of pyuria in young children with suspected UTI. Bacteriuria detected by automated urinalysis is less sensitive and specific for UTI when compared with a Gram-stained smear. We recommend using either manual or automated measurement of pyuria in combination with Gram-stained smear as the preferred technique for urinalysis of catheterized specimens obtained from children in an acute care setting.
INTRODUCTION

Urinary tract infections (UTI) are the most frequently occurring serious bacterial infection in febrile children. While urine culture is the gold standard for diagnosis of UTI, results are not available for 24-48 hours. In the interim, clinicians rely on the results of urinalysis (UA) as a screening test for preliminary diagnosis of UTI and to initiate presumptive therapy. Microscopic urinalysis includes an examination of centrifuged or uncentrifuged urine for white blood cells (WBC) and bacteria per high-powered microscopic field (hpf). However, microscopic urinalysis is subject to interpretive uncertainty from variances associated with duration of centrifugation, resuspension and technical expertise. In 1993, we described an alternative method for urinalysis, designated “enhanced urinalysis” which consists of a manual white blood cell count using a Neubauer hemacytometer and expressing the result as the number of WBC per cubic millimeter (mm$^3$) plus a Gram–stained smear on unspun urine.$^1$ Although the enhanced urinalysis is an excellent and accurate technique for screening for UTI, manual microscopy is time consuming and technologist-dependent.$^2,^3$ Subsequent to our description of the enhanced urinalysis, automated microscopy devices (flow cytometry in combination with microscopic analyzers) have replaced manual urinalysis in many laboratories.$^4-10$ Automated microscopy is less costly, less labor intensive, and faster compared with manual urinalysis. Thus, we sought to compare enhanced vs. automated urinalysis for catheterized urine specimens obtained from young children in the emergency department (ED). We hypothesized that automated urinalysis would yield similar results to the enhanced urinalysis.
MATERIALS AND METHODS

Specimen Collection and Inclusion Criteria

This prospective cohort study was conducted between January 2010 and January 2012 in the ED of a large children’s hospital in an urban setting. Standard practice when considering UTI entails performance of a midstream, clean-catch specimen from children who have urinary control and bladder catheterization for infants or children unable to void on request (most <2 years of age). During the study period, urine specimens obtained by catheter were processed by both the manual method (enhanced UA) and the automated method when adequate urine volume and technician time were available (convenience sample). The enhanced UA was interpreted and reported in real-time by a technician on duty in the hospital laboratory who was unaware of results for the automated UA. Automated UA results were batched and reviewed at a later time by a single technician (DL) unaware of results for the enhanced UA.

Urine specimens were included if they were obtained by catheterization from children suspected of having a UTI who were evaluated in the ED. Urine specimens were excluded if: (1) the child received antibiotic therapy before obtaining the urine sample, or (2) specimens were collected from children with chronic clinical conditions predisposing them to UTI or colonization of the urinary tract (e.g., spina bifida). Exclusion criteria were identified by reviewing the charts of all patients with dually processed urines. (AS, BC).

Laboratory Methods

Urine Culture: Urine specimens received in sterile packaging were cultured quantitatively.

Urine was inoculated onto 5% sheep blood agar and MacConkey agar plates with a 0.01 mL calibrated loop, incubated at 35-37°C, and examined at 24 and 48 hours for bacterial growth and colony identification.
Enhanced urinalysis: Unspun urine specimens ≥1 mL were examined microscopically for both pyuria and bacteriuria. Uncentrifuged urine was drawn into a Neubauer hemocytometer via capillary action. Pyuria was assessed by counting WBCs on each side of the chamber, averaging the value, and multiplying by 1.1 to obtain the number of WBCs per mm$^3$. Two drops of uncentrifuged urine were placed on a sterile slide within a standardized marked area of 1.5 cm diameter, air-dried, fixed and Gram-stained. Bacteriuria was assessed as the average number of bacteria per 10 oil immersion fields; morphology and Gram-stained smear results were reported.

Automated urinalysis: Automated urinalysis was performed using the IRIS iQ200 ELITE urine microscopic analyzer in tandem with the Auton Max Ax4280 automated dipstick analyzer (International Remote Imaging Systems, Chatsworth, CA). The system has the ability to process 70 specimens per hour and integrates urine microscopy and urine chemistry. It operates on the principle of flow cell digital image capture in combination with a trained neural network (Auto-Particle Recognition [APR] software). Aspirated urine is hydrodynamically focused between two layers of suspending fluid (planar flow) forcing particles to orient in a single plane facing a microscope objective lens coupled to a digital camera. Five hundred fields of digital image are captured per specimen. APR software is programmed to recognize the size, shape, contrast, and texture of urine particles. APR classifies particles into 12 categories including red blood cells (RBC), WBC, WBC clumps, hyaline casts, unclassified casts, squamous epithelial cells, non-squamous epithelial cells, yeast, bacteria, unclassified crystals, mucus and sperm. Representative images are then screened by a technician for accuracy and confirmed or adjusted accordingly.

For this study, digital images were stored for subsequent editing by a single experienced technician (DL). Dipstick results were obtained by reflectance spectroscopy and included pH, specific gravity, nitrite, and leukocyte esterase. The IRIS automated WBC count is reported per
hpf, and a conversion factor of 5.5 was used to convert the automated WBC values reported per hpf to mm$^3$ per the manufacturer. Thus, 2 WBC/hpf from the automated UA can be compared to approximately 10 WBC/mm$^3$ from the manual UA. While a difference of only 1 WBC/hpf from the automated WBC would change the predicted WBC/mm$^3$ by a factor of 5, it is important to emphasize that the report of WBC/hpf is based upon averaging 500 digital images (not merely from scanning several hpf on a slide).

**Criteria for positive UA and culture:** A positive enhanced UA was defined as $\geq 10$ WBCs per mm$^3$ and presence of any bacteria per 10 oil immersion fields on a Gram-stained smear. A positive automated UA was defined as $\geq 2$ WBCs per hpf and presence of any bacteria detected by the IRIS machine. A positive leukocyte esterase (LE) was defined as 1+, 2+ or 3+. We defined a urine culture as positive according to the American Academy of Pediatrics current clinical practice guidelines of $\geq 50,000$ colony-forming units (CFU) per mL of a single uropathogen.$^{11}$ The following organisms were considered urine pathogens: *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Citrobacter profundii*, and *Enterococcus faecalis*.

There is some suggestion that *Enterococcus faecalis* may cause less pyuria than other urinary pathogens.$^{13}$ Hence, data were also analyzed excluding the 5 patients with a urine culture positive for *E. faecalis*.

**Statistical analysis:** We analyzed data using SPSS software, and calculated sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). We also generated receiver operating characteristic (ROC) curves for the enhanced and automated WBC counts.
RESULTS

The microbiology laboratory dually processed 764 samples during the study period. We excluded specimens from 8 children with spina bifida, 5 children receiving antibiotics, and 48 specimens with incomplete information. Accordingly, 703 urine specimens were used for final analysis. Demographic information of the study population showed age range from 0 to 19 years with a median age of 10 months. Eighty-one percent (572 of 703) of subjects were <2 years old; seventy percent were female. Eighty percent of subjects (564 of 703) had a documented temperature $\geq 38.0^\circ$C in the ED or history of fever at home. Fifty-one urine specimens yielded $\geq 10,000$ CFU/mL of a single organism, 2 yielded 10,000-50,000 CFU/mL, and 49 $\geq 50,000$ CFU/mL. The prevalence of UTI defined as $\geq 50,000$ CFU/mL was 7%. Uropathogens included Escherichia coli (38), Enterococcus faecalis (5), Klebsiella pneumonia (5) and Proteus mirabilis (1).

Pyuria

Results for pyuria in urine cultures with $\geq 50,000$ CFU/mL of a single organism are shown in Table 1. The sensitivity and PPV for the enhanced UA were comparable to that for the automated UA. Results for pyuria in urine cultures yielding $\geq 50,000$ CFU/mL of a single organism excluding E. faecalis are also shown in Table 1. Sensitivity, specificity and PPV for both the enhanced UA and automated UA improved when specimens yielding E. faecalis were excluded.

Bacteriuria

Sensitivity, specificity and PPV of bacteriuria for predicting growth of $\geq 50,000$ CFU/mL of a single organism was superior for the enhanced UA compared with automated UA (Table 1). Thus, a manual Gram-stained smear clearly improved detection of bacteriuria.
When urine cultures yielding *E. faecalis* were excluded, the sensitivity of both methods improved (Table 2). The presence of any bacteria in the Gram-stained smear identified 33 (86.8%) of 38 specimens yielding *E. coli*, one specimen yielding *P. mirabilis*, 5 (100%) of 5 specimens yielding *K. pneumoniae* and 2 (40%) of 5 specimens yielding *E. faecalis*, providing further evidence that *E. faecalis* may be more difficult to detect by microscopy than other uropathogens (typically larger bacilli).

**Combined pyuria and bacteriuria:**

Using criteria of both pyuria and bacteriuria for the enhanced UA resulted in higher sensitivity and PPV as compared to values for the automated UA (Table 1). Combining automated pyuria (≥2 WBC/hpf) with a Gram-stained smear resulted in a sensitivity of 75.5% and a PPV of 84%, similar to results for the enhanced UA. Again, when urine specimens yielding *E. faecalis* were excluded, the sensitivity of both methods improved.

**Urine dipstick:**

The LE component of the dipstick UA had a sensitivity of 81.6% and a PPV of 40% for identifying urine cultures with ≥50,000 CFU/mL of a single organism. Corresponding values for the nitrite component were 36.7% and 58% in identifying urine cultures with ≥50,000 CFU/mL of a single organism.

**Receiver Operating Curves:**

ROC curves for WBC counts from both the enhanced and automated UA were constructed (Figure 1); both methods had similar area under the curve.

**DISCUSSION**

Many clinicians have been trained to interpret urinalysis results obtained by manual methods. As laboratories migrate from manual to automated methods it is important for
clinicians to understand the test characteristics of this new technology as applied to their patient population. To our knowledge, this is the first study to compare manual with automated urinalysis in children being evaluated in an acute care setting. We found that manual UA and automated UA showed similar results when comparing 10 WBC/mm³ (manual UA) with 2 WBC/hpf (automated UA). This finding is consistent with the manufacturers conversion factor of 1 WBC/hpf = 5.5 WBC/mm³. Accordingly, 2 WBC/hpf from an IRIS machine can be used as a reference standard for clinically significant pyuria. Although the IRIS threshold of 2 WBC/hpf is lower than the traditional manual microscopic threshold of 5 WBC/hpf, it should be noted that IRIS analyzes unspun urine while the traditional manual microscopic method analyzes sediment from centrifuged urine. The enhanced UA, though manual, analyzes unspun urine in a hemocytometer and is more directly comparable to the IRIS UA. The importance of detecting clinically significant pyuria is highlighted in the recent AAP practice guidelines which state that the diagnosis of UTI requires both urinalysis results suggesting infection (pyuria and/or bacteriuria) and presence of at least 50,000 CFU/ml of a uropathogen cultured from a urine specimen obtained through catheterization or suprapubic aspiration.¹¹

The ability of the automated UA to detect bacteriuria was inferior to the enhanced UA (sensitivity 73.4% vs. 83.6%). The difference in performance for bacteriuria may result from either misclassification of unstained particles (cells, casts, or debris) using the automated computer algorithm or enhanced visibility of bacteria using the manual Gram-stained smear. The higher specificity of the Gram-stained smear (95.7% vs. 84.5%) suggests that staining facilitates a more accurate discrimination between bacteria and debris. E. faecalis appears difficult to detect
by either automated or Gram-stained smear techniques. Of note, we had no specimens with *Staphylococcus saprophyticus*, which we suspect may be equally difficult to detect.

At Children’s Hospital of Pittsburgh, based on our previous work, the enhanced UA is used as the preferred method for analysis of urine specimens obtained by catheter.\(^1\,\,^{22}\) The present study showed results similar to those reported by Shaw et al.\(^23\) but a slightly lower sensitivity of the enhanced urinalysis compared to our previously published experience in a larger sample. In the initial study, we sampled all children with catheter-obtained specimens (similar to this study) and reported a sensitivity for pyuria of 84.5%,\(^1\) whereas in our second study--limited to febrile children less than 2 yo--we reported a sensitivity of 91%.\(^22\) Thus, differences in risk factors such as age, race, and temperature should be considered when (1) comparing published reports, (2) interpreting UA results, and (3) generalizing these results to other clinical practices. In the present report, we chose to examine results from all children in whom a catheterized urine specimen was obtained, rather than a more narrowly defined population (e.g. children with documented fever), because our current practice is to perform an enhanced UA on all catheterized specimens. This design allowed us to compare the enhanced UA with the automated UA under the real-world clinical conditions in which enhanced UA is ordered in our ED.

Two technologies are currently available for performing automated UA in uncentrifuged specimens. The UF series of instruments by Sysmex TOA Medical Electronics (Hamburg, Germany) uses fluorescent staining to sort and identify urinary particles.\(^7\,\,^9\,\,^{15–20}\) In contrast, the IRIS technology used in this study uses digital imagery followed by APR and editing to sort and
identify urinary particles. Both methods show high correlation with manual counting of WBCs. However, identification of bacteriuria is less reliable. Most studies have been performed in adults, in unselected specimens, or in specific clinical settings (urology or nephrology clinics) that limit generalizability to our patient population. In a pediatric population attending a nephrology or urology clinic, Lunn et al examined 186 samples using the UF Sysmex technology and reported a sensitivity of 89% for identifying UTI.

This study has several limitations. The use of a highly trained single technician for interpretation of the automated UA specimens might inflate accuracy compared with results obtained from a larger sample of technicians with less experience and training. Similarly, we evaluated results from one specific instrument, the IRIS iQ200 ELITE; accordingly, our results may not be generalizable to other automated instruments. Although we postulate that automated urinalysis is less costly than manual urinalysis, a formal cost analysis including all costs of labor, instruments, supplies, and maintenance with adjustments for volume and depreciation is beyond the scope of this report. Also, results obtained using urine specimens acquired by catheterization from children in an ED-setting, may not be generalizable to populations with higher or lower prevalence of UTI. Lastly, unlike the new AAP definition, our definition of UTI did not require pyuria because it is not possible to include pyuria in the definition while simultaneously measuring the sensitivity of pyuria. As a consequence, we may have included some children with asymptomatic colonization of the urinary tract. Colonization occurs in approximately 1.5% of young children. Patients with colonization would be misclassified in this study as “missed” UTI and would artificially lower the sensitivity of pyuria.
Conclusion:

Automated UA is comparable to the manual UA for detection of pyuria in children with suspected UTI in an acute care setting. In contrast, bacteriuria detected by automated UA is less sensitive and specific for diagnosing UTI than a Gram-stained smear. We recommend using either manual or automated measurement of pyuria in combination with a Gram-stained smear as the preferred technique for UA of specimens obtained by catheter from children in the acute care setting.

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Figure Legends

**Figure 1.** Receiver Operating Curves (ROC) for enhanced and automated WBC counts.

The area under the ROC curve for enhanced urinalysis is 0.89. The area under the ROC curve for automated urinalysis is 0.91.