New Generation IQ-200 Automated Urine Microscopy Analyzer Compared With KOVA Cell Chamber

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Objective: The examination of the urine remains to be one of the most commonly performed tests in laboratory practice. Currently, laboratories also need to accredit their urine diagnostics by comparing their measurement methods to acceptable references. In this study we compared particle counts obtained by new generation automated technique, image capture analysis (IQ-200) with those of a standardized chamber counts. **Design and Methods:** The same 258 urine samples from different departments of a hospital assayed by IQ-200 were analyzed in parallel with the KOVA cell chamber system. Clinically significant discrepancy results (positive vs. negative) for red blood cell (RBC) and white blood cell (WBC) were also compared with

those obtained by dipstick testing. **Results:** There was a good agreement between the automated system and sediment microscopy for RBCs, WBCs, and squamous epithelial cells (SCs) ($r=0.90$; $r=0.80$; $r=0.72$, respectively: $P<0.001$). The IQ-200 was more sensitive for determining RBCs, WBCs, and SCs than other formed elements. **Conclusions:** IQ-200 can perform accurate quantification of microscopic element in urine. However, automated techniques are not completely free of error. Therefore, by adopting an appropriate algorithm and combining the results with strip analysis and other laboratory tests allows further reduction of clinically important errors. J. Clin. Lab. Anal. 24:67–71, 2010. © 2010 Wiley-Liss, Inc.

Key words: automated analyzer; chamber counting; urine analysis

INTRODUCTION

Manual microscopic urine sediment analysis has been the traditional reference method of urine sediment analysis. Furthermore, the microscopic examination of urinary sediment is labor intensive and has poor precision owing to variations in sediment preparation and counting technique (1). Guidelines prepared by the National Committee for Clinical and Laboratory Standards (NCCLS) and European Urinalysis Group of the European Confederation of Laboratory Medicine (ECLM) to improve standardization with use of an automated systems or manual microscopy (2,3). To improve standardization with use of an automated system or standardized procedure to count the cells in a chamber within a predefined volume NCCLS and European Confederation of Laboratory Medicine published guidelines (2,3). To automate microscopy, a new generation

analyzers, based on different technologies, has been developed (4,5).

Recently, IQ-200 automated urine microscopy analyzer (IQ-200 Iris Diagnostics, USA), an automatic particle recognition performance, has been introduced. The IQ-200 uses digital imaging to capture and analyze 500 photos from each sample and Auto Particle Recognition software (APR) to classify the isolated images of the urine constituents into 12 categories and report quantitatively (5). It has not been shown

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previously whether the IQ-200 is sensitive for all urine particles.

In this study we evaluated by comparing the results with those from manual microscopy using standardized counting chambers.

MATERIAL METHODS

Urine Samples

Specimens were obtained from the outpatients and inpatients admitted to Dokuz Eylul University Hospital. Our institution is a 900-bed University hospital with 58,500 inpatient and 600,920 outpatients in 2008 from whom 69,800 routine urinalysis tests were ordered in a one-year period. Of this, 300 freshly collected urine samples from different departments of the hospital (190 women aged 4–79 years; 110 men aged 5–76 years) were randomly selected for this study. Every other fifth sample admitted into the laboratory was enrolled into the study group. All specimens were examined within an hour of receipt in the laboratory. Forty-two samples that needed dilution for manual microscopic examination because of very large cell numbers were excluded from the correlation study.

Finally, 42 results were not used for the comparison method; thus, 258 (86%) remained in the evaluation study.

Standardized Sediment Microscopy

The manual microscopic sediment examination was performed according to NCCLS guidelines (2). We used the standardized KOVA cell chamber system for microscopic counting (6). After automated counting of particles by IQ-200 as described by the manufacturer (5), each urine sample (10 mL) was centrifuged at 400g for 5 min, and 9 mL of the supernatant was removed. The pellet was mixed well and one drop was placed in the KOVA cell chamber.

For counting in the KOVA chamber, the middle section in the grid was chosen and urine particles were counted at a 400 $\times$ magnification by bright field microscopy. All microscopic evaluation was performed by the same experienced technologist.

IQ-200

Imaged-based analyzer IQ-200 uses planar flow cytometry and analyzes the particle images of uncentrifuged native urine utilizing Auto Particle Recognition software, a trained neural net that differentiates particles by texture, contrast, size, and shape. This system classifies urine constituents into 12 particle categories and quantitatively report concentrations red blood cells (RBCs), white blood cells (WBCs), WBC clumps (WBCCs), hyaline casts, pathological casts (or

unclassified casts), squamous epithelial cells (SCs), nonsquamous epithelial cells (NSE; combination of renal tubular cell and transitional cell), bacteria, yeasts, crystals, and sperm. The results can be auto-reported based on manufacturer-defined criteria. Certain classes of particles (pathological cast, crystals, and NSE) may be subclassified under operator control (5). In this study all results were reviewed and edited, if necessary, by a trained operator.

Criteria for Positive Result

The results for RBCs, WBCs, and SCs were expressed as the number of cells per microliter of urine and those for other formed elements (SRC, cast bacteria, yeast, and crystal) were evaluated qualitatively.

Clinical cut-off values for formed elements reported as the number of element per microliter urine sample were as follows: RBC < 20/ μ L, WBC < 25/ μ L, SCs < 30/ μ L. Positivity limits for the particle evaluated qualitatively were those recommended by the manufacturers. For the strip, a positive urine sample was considered to be more than traces for blood (hemoglobin) and leukocytes (leukocyte esterase reaction).

Technical Performance

Within-run imprecision was evaluated by two urine pools with high (mean = 202 RBC/ μ L, 218 WBC/ μ L, 40 EC/ μ L) and low (mean = 1 RBC/ μ L, 3 WBC/ μ L, 8 EC/ μ L) cell concentrations. Each urine sample was analyzed a total of 20 times during a single day. For between-run imprecision commercial IQ-200 negative (0–4 RBC cell/ μ L) and positive (855–1117 RBC cell/ μ L) quality control samples were used as supplied by the manufacturers.

To assess sample dependent carry-over low (L) pools (mean = 1 RBC/ μ L) and high (H) pools (mean = 935 RBC/ μ L) analyzed sequentially in order L-L-L-H-H-L-HH-L-L-L-L-H-H-L-H-H-L-H-H-L. The results of five low samples following low samples were compared with five low samples following the high samples.

Statistics

All data were collected on Microsoft Excel spreadsheets. Chamber counts were entered manually. Results of analyzer and dipstick were obtained from laboratory information system. Statistical analysis was performed using SPSS for windows version 11.0 (SPSS Inc., Chicago, IL). Method comparison for WBCs, RBCs, and SCs was performed using linear regression analysis.

Diagnostic accuracy for all particles was calculated as the fraction of true and false classified cases according to

reference method. In all calculations, manual microscopy was used as the reference method.

RESULTS

Precision Study

For low pools, intra-assay coefficient of variation (CV) was 3, 2, and 5% for RBC, WBC, and EC, respectively. For high pool, intra-assay CV was 2, 2, and 4% for RBC, WBC, and EC, respectively. Inter-assay CV was 3% for negative control and 2% for positive control.

Carryover

Carryover was calculated as less than 2%.

Comparison Study

In total, 300 specimens were examined by the analyzers and manual microscopy. We included 258 results for the comparison study.

Comparison of results obtained by sediment microscopy and automated system showed a good agreement for RBCs, WBCs, and SCs ($r = 0.9$; $r = 0.8$; $r = 0.72$, respectively for IQ-200, $P < 0.001$). The regression equation by IQ-200 (y) and Kova chamber (x) of urine samples was $y = 0.98x + 5.8$ RBC/ μ L, $y = 0.7x + 6.3$ WBC/ μ L, and $y = 0.69x + 7.4$ SC/ μ L. The normal P-P plots (Figs. 1–3) indicate that the data set is normally distributed.

Clinically significant discrepancy results (positive vs. negative) for RBCs and WBCs were also compared with those obtained by dipstick testing. There were 36 (14%) RBC and 40 (15%) WBC cases of clinically significant discrepancies for IQ-200. Comparing these discrepancy results with those of dipstick we found that 17 of RBC

samples (6% of total) and 23 of WBC samples (9% of total) agree with dipstick and manual microscopic methods identifying the sample as normal/abnormal.

Diagnostic accuracy, relative to manual microscopy for IQ-200, is shown in Tables 1 and 2. Of the 258 (40%) samples tested, 103 were reportable without any

Fig. 2. WBCs measured by IQ-200 compared with kova system.

Fig. 3. SCs measured by IQ-200 compared with kova system.

Fig. 1. RBCs measured by IQ-200 compared with kova system.

TABLE 1. Analytic Comparison of Measuring Urine Cells by IQ-200 vs. Kova

	Urinary RBCs	Urinary WBCs	Urinary SCs	Urinary NSE
Sensitivity	80	70	72	71
Specificity	87	97	96	94
PPV	73	93	90	76
NPV	94	87	82	89

Data are given in percentages. ppv, positive predictive value; npv, negative predictive value.

TABLE 2. Analytic Comparison of Measuring formed elements by IQ-200 vs. Kova

	Hyaline casts	Pathologic casts	Yeasts	Crystals	Bacteria
Sensitivity	55	50	61	74	70
Specifity	97	99	95	97	94
PPV	64	66	52	86	70
NPV	96	92	97	95	94

Data are given in percentages. ppv, positive predictive value; npv, negative predictive value.

additional operator classification and 155 samples were edited by operator.

DISCUSSION

Because of differing measurement principles, different results can be expected between urinalysis methods (7). Several comparison methods have been used in the evaluation of automated systems (8,9). First, the Yellow IRIS urinalysis instrument has automated the microscopic evaluation of urine sediment. Several studies have demonstrated that it has increased precision over routine manual urinalysis, and that it can detect more abnormalities than conventional microscopy (10,11). In our study we used standardized procedure to count the cells in a chamber as recommended by the NCCLS. Though we found good correlation for major formed elements (RBCs, WBCs, and SCs) automated systems tended to give larger values as in other studies (12,13). The reason for these differences in cell counting is unclear. It can be concluded that manual microscopic technique has several methodological steps that include centrifugation and resuspension steps that are either incomplete or lead to cellular loss and lysis cell counts than microscopy (13,14). We see that differences for manual and automated results was much more evident in urine samples with large particle (bacteria, crystal, and yeast) and nonformed elements. These particles can influence the classification of cells by IQ-200. In some cases both yeast and small calcium oxalate crystals were incorrectly classified as RBCs. We suggest that samples containing these particles must be examined in more detail for true classification of IQ-200 and checked microscopically. Also, strip results were not included in all comparison study. So reducing the error rate could be achieved by cross checking the analyzers' result with dipstick data such as RBC vs. blood, WBC vs. leucocyte esterase, cast vs. protein, bacteria vs. nitrite as recommended by the manufacturer. To our observation visual images of major cellular elements (RBCs and SCs) by IQ-200 were similar with manual microscopy except for

WBCs. For this reason these cells could be easily recognized on the screen, but in some cases WBCs classified as artifact were difficult to interpret by the technologist, especially WBCCs, disintegrating and atypical WBC. On the other hand, in some samples, partial images of WBC and NSE by IQ-200 may sometimes be missclassified by APR software and operator.

In some cases, transitional and renal epithelial cell counts showed differences between two techniques. Since some of the transitional and renal cells are incorrectly classified as artifact, the operator needs to review the artifact for true classification.

Bright field microscopy of unstained preparations allows only poor visualization of hyaline casts (15). The detection of casts (hyaline and pathologic) is less definitive than is detection of cells by analyzer. Comparison with manual microscopy demonstrated a high number of false-negative count by analyzers. Casts were often shown in the artifact category by IQ-200. Artifact must always be checked to get true positive results for casts. Similar findings have been reported by the Yellow IRIS and 900 UDX (9,16). The difference in detection of casts may be due to the difference in volume and concentration of urine sample analyzed. Perhaps because of our patients' selection we found cast of only 6% of samples by sediment microscopy. This number is too low for detailed discussion. We therefore propose that study should be performed on samples from patients with renal disorder to evaluate the reliability of the analyzers in detecting cast.

Finally, we conclude that IQ-200 and standardized chamber counts perform equally well in detecting main cell count. However, automated system is not completely free of error. When all these parameters are evaluated in combination with clinical signs, physical examination, thorough history, and other laboratory tests, a diagnosis will often be attained.

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