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Is automated urine sediment analyzer iQ200 a practical replacement ?

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Background : *Short turn around time and precision are essential for microscopic examination of urine sediment which is a complicated and time-consuming procedures. We evaluate compatibility of analytical performance between an automated urine analyzer and routine traditional manual methods in order to verify the applicability of automated urine analyzer in our laboratory.*

Method : *Three hundred and forty-eight urine samples collected from pediatric renal clinic(two hundred samples) and routine check-up programs (one hundred and forty-eight samples) were evaluated by both iQ200, an automated urine analyzer, and routine manual methods. The results of which were reported in ranges and comparatively analyzed.*

Result : *Optimal agreement between both reports yielded in almost all parameters including WBC (Kw = 0.86), RBC (Kw = 0.86), squamous epithelium (Kw = 0.82), broad granular cast (Kw = 0.90), calcium oxalate crystal (Kw = 0.81) and uric acid crystal (Kw = 0.83) while substantial agreement for bacteria detection was seen (Kw = 0.63) and only moderate agreement for bladder epithelium (Kw = 0.43) and budding yeast (Kw = 0.46).*

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Conclusion : *Automated urine analyzer has overall excellent agreement with routine manual methods with exception in a few parameters. Crosschecking with urine chemistry analysis, selective reviews and reclassification by experienced technicians are strongly suggested. However, organized selection regarding when, where and by which means should be implicated since it is believed to be the most practical solution.*

Keywords : *Automated urine analyzers, iQ200.*

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ภาฤทธิ์ เมฆอรุณกมล, พจณิชา ลิยมาสวัสดิ์, ประภาวดี เอกวงศ์, นิศาชล เทพา, ชุติธร เกตุลอย, พรรธนมณฑน์ อุชชิน. เครื่องตรวจตะกอนปัสสาวะอัตโนมัติ iQ200, ทางเลือกใหม่ที่ใช้ได้จริงหรือไม่?. จุฬาลงกรณ์เวชสาร 2552 พ.ย. - ธ.ค.; 53(6): 477 - 87

ที่มา : *ความแม่นยำและระยะเวลาการรอคอยที่สั้นเป็นสิ่งที่สำคัญยิ่งต่อการตรวจตะกอนปัสสาวะในห้องปฏิบัติการ อย่างไรก็ดีจุลทรรศน์ศาสตร์ของการตรวจตะกอนปัสสาวะนั้น มีกระบวนการที่ซับซ้อนและใช้เวลานาน ในการศึกษานี้ผู้นิพนธ์จึงได้ศึกษาเปรียบเทียบ ผลวิเคราะห์ตะกอนปัสสาวะระหว่างวิธีจุลทรรศน์ศาสตร์และเครื่องอัตโนมัติ เพื่อประเมินถึงความเป็นไปได้ในการนำเครื่องอัตโนมัติมาใช้ในการตรวจตะกอนปัสสาวะในห้องปฏิบัติการ*

วิธีการ : *ทำการเปรียบเทียบผลวิเคราะห์ที่รายงานเป็นช่วงจำนวนตะกอน ที่ได้จากวิธีจุลทรรศน์ศาสตร์และเครื่องอัตโนมัติ โดยใช้ตัวอย่างปัสสาวะ 348 ตัวอย่างที่เก็บจากผู้ป่วยกุมารเวชศาสตร์โรคไตและผู้มาตรวจร่างกายในโครงการตรวจสุขภาพประจำปีของโรงพยาบาลจุฬาลงกรณ์*

ผลการศึกษา : *พบค่าความสอดคล้องระดับสูงสุดระหว่างผลการตรวจวิเคราะห์ทั้งสองวิธี ในค่าชี้วัดเกือบทุกชนิดได้แก่ เม็ดเลือดขาว (Kw = 0.86), เม็ดเลือดแดง (Kw = 0.86), เซลล์เยื่อบุชนิด squamous (Kw = 0.82), ตะกอน broad granular (Kw = 0.90), ผลึก calcium oxalate (Kw = 0.81) และผลึกกรดยูริก (Kw = 0.83) มีเพียงแบคทีเรียที่พบค่าความสอดคล้องระดับสูง (Kw = 0.63) โดยเซลล์เยื่อบุผิวกระเพาะปัสสาวะและยีสต์พบค่าความสอดคล้องระดับกลางเท่านั้น (Kw = 0.43 และ 0.46 ตามลำดับ)*

สรุปผล : *เครื่องตรวจตะกอนอัตโนมัติมีความสอดคล้องที่ดีเยี่ยมกับวิธีการตรวจทางจุลทรรศน์ศาสตร์ในค่าชี้วัดส่วนใหญ่ อย่างไรก็ดีการนำเครื่องมือดังกล่าวมาใช้ในห้องปฏิบัติการนั้น ควรมีผู้เชี่ยวชาญทำหน้าที่จัดหมวดหมู่และทบทวนประมวลภาพตะกอนจากเครื่องก่อนการรายงานผลด้วย โดยผู้นิพนธ์เสนอว่าการประยุกต์ใช้แต่ละวิธีในบริบทที่เหมาะสมน่าจะเป็นวิธีที่ดีที่สุด*

คำสำคัญ : *เครื่องตรวจตะกอนปัสสาวะอัตโนมัติ, iQ200*

Microscopic examination of urine sediment plays an essential role in urinalysis since it can give invaluable information regarding renal and urinary tract pathology.[1-3] However, several laboratories are challenged with its experienced technicians-requiring, time-consuming and labor-consuming procedures as the inter-operator discrepancy remained a questionable threat to the precision.[1,4] Moreover, due to increasing amount of requests especially in large hospitals, short turn around time becomes more essential.[4, 5]

Therefore, new technologies in automated urine microscopy have been developed in attempt to solve the limitations of manual microscopic technique in order to improve precision, accuracy, speed of throughput and to eliminate multi-step procedures which subsequently save labor and time.

The aim of this study was to evaluate the compatibility of analytical performance between an automated urine analyzer and routine traditional manual methods in both pediatric patients with nephrological abnormalities and assumable healthy people from routine check-up programs. Excellent agreement among most of parameters were obtained, except for bacteria and budding yeast.

Material and Methods

Specimen Collections

The total of 348 freshly collected urine specimens from the out patient laboratory at King Chulalongkorn Memorial Hospital during a 12-week period were submitted for the study. The majority of the samples were voided from pediatric renal clinic (200 samples) and the remainder from routine check-up programs of the hospital (148 samples), thus including a variety of abnormalities to provide a wide range of results on the formed elements analysis. Clean containers were used for urine collection. All submitted specimens contained more than 15 ml of urine to ensure adequate volume for the study.

The study has been approved by ethics committee of the Faculty of Medicine, Chulalongkorn University.

Manual microscopy

The routine conventional urinalysis method was performed according to King Chulalongkorn Memorial Hospital's work instruction. Briefly, ten milliliters of well-mixed urine was centrifuged in 15 ml conical test tube at 1,500 rpm. Then 9 ml of the supernatant was discarded and the remaining was re-suspended giving 10 times concentrated urine, after which a drop of the specimen was inspected on a glass slide under 22 x 22 mm cover slip at x 400 magnification by light microscope. The formed elements were counted, averaged and reported in ranges per HPF. To reduce inter-observer variation, one technician performed all the manual urinalysis, while another performed all the automated ones. All procedures were completed within two hours after specimen collection.

Automated urine analyzer

iQ200 (IRIS, Chatsworth, California, USA), an automated image-based urinalysis system, was used in this study. With a planar flow imaging technique and Automatic Particle Recognition software (APR, IRIS Diagnostics), the system classified and quantified cellular particles in native un-centrifuged urine into 12 categories plus artifacts. The device required at

least 3 ml of urine for analysis. Calibration was performed at the beginning of the study and on each day during the study. iQ Focus, iQ negative and iQ positive control samples were run according to the manufacturer's instruction. Calibrator, focus and positive controls are suspensions of fixed human red cells in a particle free buffer, while negative control is a particle free buffer solution.

During the analytical process, the instrument aspirated approximate 1 ml, two microlitres of which was used for analysis. Elements in the urine were captured using a CCD digital camera imaging in a planar flow cell between hydrodynamic focusing sheath fluid from which 500 frames per sample were yielded. Afterward, APR neural network would categorize each captured particle based on its shape, size, contrast and texture features. The device was set to display all captured images on screen for the technician to review and reclassify before reporting, if necessary. The report from the automated instrument was set to be reported in the same ranges as that of the manual method.

Precision study

As for within-run reproducibility, 20 times repeated measurements of two specimens selected from low pool and high pool of RBC and WBC by automated means were conducted within one day. The precision of the device was assessed by the coefficients of variation (CVs) calculated.

As for between-run imprecision, due to storage limitation of urine samples, negative and positive controls provided by the manufacturer were used. Both controls were run each day for 35 days during the study period; the result of which were calculated and expressed as coefficients of variation (CVs).

Linearity and carry-over Assessments

Linearity of counting was assessed by four-fold serial dilutions of 20 urine specimens by the manufacturer's instruction. Each dilution was analyzed by the automated instrument and the results of which were then compared with the expected values. The slope and interception were determined.

Carry-over test was performed using iQ positive control with fixed concentration of RBC followed by iQ negative solution. The test was serially run everyday before the study.

Statistical Analysis

Since the reported ranges were all in ordinal scale, therefore linear weighted kappa coefficient was used.[6] Sensitivity, specificity, negative and positive predictive value were calculated using Microsoft Excel Spread Sheaths (Microsoft Corporation).

A p-value of 0.05 was used as the limit of statistical significance, which yielded a 95 % binomial confidence interval of proportion. The linear weighted kappa coefficient more than 0.8 was considered optimal agreement, 0.61 - 0.8 was considered substantial agreement, 0.41 - 0.6 was considered moderate agreement, and $\leq$ 0.4 was considered poor agreement.[6]

Correlation studies for all 348 urine samples were conducted by cross tabulating all data result reported in ranges to calculate agreement between two methods. Sensitivity, specificity and linear weighted kappa coefficient were yielded for each parameters obtained. Confidence intervals for

proportions are calculated according to the Wilson efficient score method corrected for continuity.

Result

As for quality control by within-run reproduction of two samples by the automated analyzer,less coefficient of variation in the series of samples from the low pool was demonstrated. This expression was seen both for RBC and WBC (Table 1).

As for between-run imprecision, iQ positive and negative control were used. The result of positive control was in range of 862 -1075 cells/uL (1018.97±57.25 CV = 5.62%), while negative control's was in the range of 0 - 20 cells/uL (2.51±3.97 %CV = 158.02%).

Linearity was obtained by comparing the results of four-fold serial dilutions of 20 samples and the expected value of which. Pearson correlation was conducted, from which yielded rho(r) of 0.991 for WBC and 0.996 for RBC as depicted in Fig. A and B.

Table 1. Mean and standard deviation from within-run reproduction by the automated analyzer.

		Sample1	Sample2
WBC	Cell range	1-3	310-431
	Mean+SD	1.96+0.99	337.35+42.77
RBC	Cell range	0-1	5-10
	Mean+SD	0.38+0.28	7.35+1.60

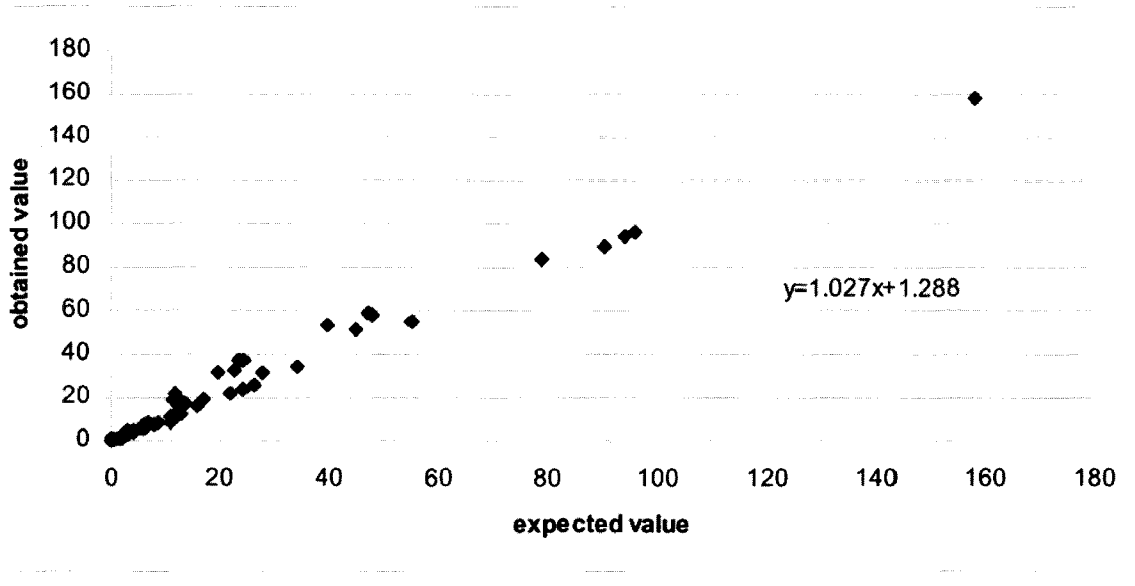

Figure. A Linearity assessment of WBC value obtained from four-fold serial dilution by the automated analyzer.

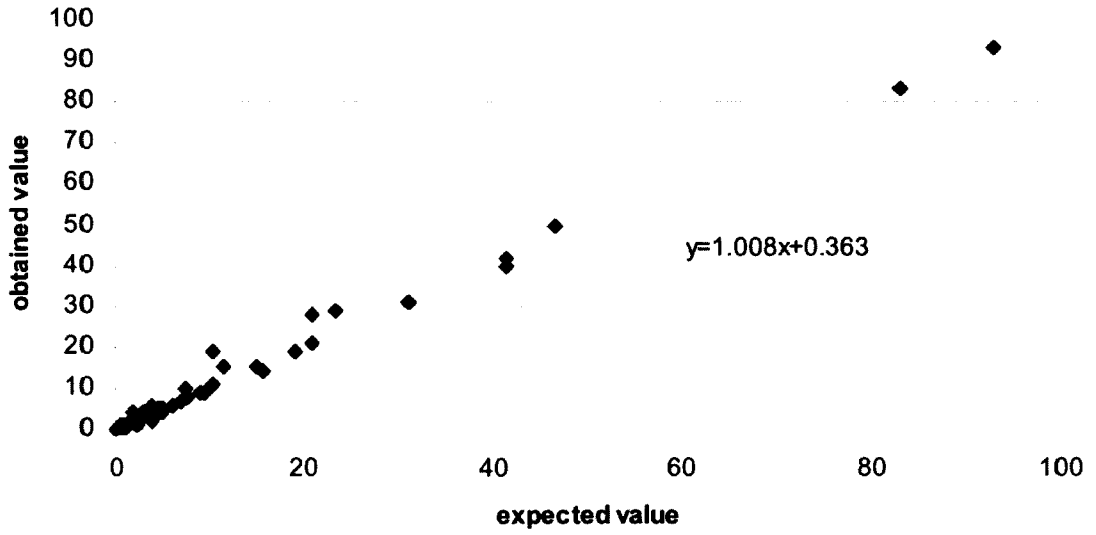

Figure B. Linearity assessment of RBC value obtained from four-fold serial dilution by the automated analyzer.

No carry-over was found during the study.

As for WBC and RBC counts, the correlations were optimal. There were 80.46% and 78.45% absolute agreement and 97.70% and 95.69% agreement within one rank respectively. Linearly weighted, the cross tabulation yielded a kappa coefficient of 0.8614 for CBC and 0.8606 for RBC. There was one WBC clump reported by the automated analyzer which agreed with manual method's report.

As for squamous epithelium, 87.93% were in the same rank and 98.28% agreed within one rank, yielded linearly weighted kappa coefficient (Kw) of 0.8217 (95% CI 0.7657 - 0.8777). The proportions of agreement were highest in the range of 0-1 cell/HPF (0.9244; 95% CI, 0.8862 - 0.9509).

As for bacteria and budding yeast, the used scales were few, 1+, 2+ and 3+. Of the bacteria results, 81.03% were in the same rank, and 96.84% agreed within one rank. Regrading the budding yeast, 98.56% were in the same rank and 99.71% agreed within one rank. Kw of 0.6341 (95% CI 0.5519 - 0.7163) and 0.4567 (95% CI 0.0399 - 0.8735) were obtained, respectively. Highest proportions of agreement were both seen in negative results (0.8023; 95% CI = 0.7474 - 0.8481 for bacteria and 0.9884; 95% CI 0.9686 - 0.9963 for budding yeast).

Non-squamous epithelium reported by iQ 200 was further reviewed and subcategorized into bladder epithelium and broad transitional epithelium by the technician. However, there was only one positive specimen for broad transitional epithelium. It was then excluded from the analysis.

The remaining specimens positive for bladder epithelium were calculated, from which yielded: Kw of 0.4329 (95% CI 0.3109-0.5549). Eighty-eight and fifty-one hundredths percent (88.51%) were in the same rank, and 99.43% agreed within one rank. Proportions of agreement were highest in negative result (0.8936; 95% CI = 0.8539 - 0.9238).

There were three classes of casts detected: hyaline cast, broad granular cast and convoluted cast. Nevertheless, we did not include convoluted cast in the analysis due to very few number of positive results (only two positive specimens were reported).

As for broad granular cast, Kw was 0.9078 (95% CI = 0.8308 - 0.9848). Ninety-nine and forty-three hundredths percent (99.43%) were in the same rank and there was perfect agreement (100%) within one rank of all results. Accordingly, the proportions of agreement was also perfect in negative result (proportion = 1; 95% CI = 0.9861-1) and proportion in 0-1 range was 0.8333 (95% CI = 0.3648 - 0.9912).

As for hyaline cast, Kw was 0.8139 (95% CI = 0.719 - 0.9088) with perfect proportion of agreement in negative result (1; 95% CI = 0.9855-1). 97.41% were in the same rank and 99.14% agreed within one rank.

There were two classes of crystal sediments detected, namely: calcium oxalate crystal and uric acid crystal; both of them yielded high Kw of 0.8055 (95% CI 0.6744 - 0.9366) and 0.8309 (95% CI 0.6362-1), respectively. Moreover, the highest proportion of agreements were seen in negative results of both classes (0.9908; 95% CI = 0.9712 - 0.9976 and 0.9971; 95% CI = 0.9813 - 0.9998).

As for calcium oxalate crystal, 97.41% were in the same rank, and 99.14% agreed within one rank. As for uric acid crystal, 99.43% were in the same rank, and all results agreed within one rank.

Comparing to the conventional manual method, high sensitivity, specificity, efficiency, negative predictive value and positive predictive value were obtained in almost all parameters from iQ 200 with only few exceptions,which are sensitivity of bacterial detection (66.67%), sensitivity of budding yeast detection (50%) and positive predictive value of budding yeast detection (50%) as described in Table 2.

Table 2. sensitivity, specificity, efficiency, negative predictive value (NPV) and positive predictive value (PPV) of all parameters by iQ200 in comparison with conventional manual method.

	Number of positive result	Cut-off of positivity	Sensitivity (%)	Specificity (%)	Efficiency (%)	NPV (%)	PPV (%)
WBC	66	5	91.67	95.83	95.40	99.01	71.74
RBC	54	5	92.54	98.98	97.99	98.64	94.34
Bacteria	135	few	66.67	97.18	85.34	82.14	93.75
Squamous epithelium	22	5	93.75	98.80	98.56	99.70	78.95
Bladder epithelium	19	1	100.00	89.36	89.94	100.00	35.19
Budding yeast	11	few	50.00	99.42	98.85	99.42	50.00
Broad granular cast	16	4	100.00	100.00	100.00	100.00	100.00
Hyaline cast	11	2	100.00	100.00	100.00	100.00	100.00
Calcium oxalate crystal	24	few	87.50	100.00	99.14	99.08	100.00
Uric acid crystal	12	few	83.33	100	99.71	99.71	100

Besides formed elements mentioned earlier, there was also other sediment detected in the study but the number of positive results was too small to be included in the analysis. These particles were oval fat body (3 positive results), convoluted cast (2 positive results), broad fatty cast (1 positive result), broad transitional epithelial cast (1 positive result) and spermatozoa (2 positive results).

Moreover, there were numbers of specimens reported concerning mucous and amorphous elements. Considering that they had no clinical significance, their data were therefore not analyzed.

Discussion

Despite many variations in conventional microscopic methodologies of urinalysis, the system is still widely and routinely used in many laboratories with commonly acceptable results. Optimal agreement (Kw > 0.8) were obtained in almost all parameters including WBC, RBC, squamous epithelium, broad granular cast, calcium oxalate crystal and uric acid crystal. However, substantial agreement was seen in bacteria detection and only moderate agreement for bladder epithelium and budding yeast.

Poorer agreement in these three parameters was similarly reported by T.I. Chien *et al.*, Lamchiangdhase *et al.*, Alves *et al.* and Linko *et al.*.[1, 7-9] This difference might be due to limited ability of iQ 200 in detection of bacteria other than rod forms.

In spite of optimal agreement in casts and crystals result, numbers of reclassifications had been performed by the technician before submitting the results. On the other hand, there was no image needed to be reclassified after reviewing of the stored images of RBC, WBC and squamous cell epithelium. However, these three parameters yielded just as high agreement as others indicating higher diagnostic ability.

Highest Kw and efficiency was seen in broad granular cast parameter. The reason of which could be that broad granular casts have unique size, shape, contrast and texture thus prominently stood out among others by APC classification.

The proportions of agreement of all parameters were highest in the lowest rank. This result is well correlated with high NPV and specificity calculated. This may imply more reliability on negative result; however, small numbers of positive samples (<15) of the same parameters (budding yeast, hyaline cast, uric acid crystal) could limit this interpretation.

In our study, although moderate to optimal agreement was seen in all parameters but proportions of agreement also decreased in higher rank of positive detection. Moreover, all results from iQ 200 submitted in this study were post-review by the technician therefore, we concluded that the iQ 200 had quite comparable result with manual microscopy and the automated device could potentially be an effective screening tool thus increasing throughput and precision of urinalysis however,a review by trained technicians is required especially on highly positive or complicated results. It is expected that more reliability and higher agreement could be achieved by combination with urine chemistry analysis (i.e. WBC vs leukocyte esterase, bacteria *vs.* nitrite, cast *vs.* protein etc.) to crosscheck with automated report and help the technician to properly select which samples should be submitted for microscopic reevaluation.

The great advantage of the automated urine analyzer is the high speed throughput (70 tests per hour and up to 101 tests per hour for Elite iQ200 and iQ200 sprint) comparing to multi-step time consuming manual method.[10] Moreover, the images storing ability can be very beneficial in some complicated samples, for which repeated examinations by other technicians may be required.

As for research-based laboratories, although high throughput and short turn around time are not crucially necessary, but images-storing system of iQ200 can enable researchers to reevaluate questionable results and to eliminate inter-observer variability giving more consistent results and more advantages than manual methods .

Since cost-effectiveness is quite a considerable issue, manual method is routinely used in our institution.[4] However, when workload increased and microscopic reevaluation was not highly anticipated such as in routine physical check up programs or for renal clinic where complicate results were expected, the automated urine analyzer would be used. We believe that a combination of both manual and automated methods and organized selection of when and where should which means be implicated are the most appropriate approach to improve work flow in urinalysis laboratory.

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