An image-based automated analysis of urine sediment

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Laboratories of Medirex Company employ automated analysers for urine determination realized via on-line combination of image-based automated urine sediment analyser (Iris iQ200) and dip-stick chemistry analyser (Aution Max AX-4030). This article discusses sample assessment with respect to everyday routine, maintenance of the analyser, precision study of Iris iQ200, and interpretation of possible interferences. Sample assessment includes general sediment assessment, reviewing of flagged samples with respect to results from chemical analysis. Due to the use of Auto-Particle Recognition software, several discrepancies or misclassifications can occur as a consequence of disintegration, morphological changes of cells, etc. However, work station enables the manual reviewing of the images before the results are sent to a laboratory information system. Not only to prevent discrepancies, but also to provide accurate and precise results, manual reviewing by trained laboratory technicians is performed considering urine sediment and chemical properties when available.

Key words: urine sediment, automated urinalysis, manual microscopy, Iris iQ200 interference

Automatizovaná analýza močového sedimentu

Vyšetrenie močového sedimentu patrí medzi základné laboratórne vyšetrenia. Spracovanie veľkého množstva prijatých vzoriek moču v priebehu jedného dňa si preto vyžaduje automatizovanú analýzu. Z toho dôvodu sú v laboratóriách spoločnosti Medirex, a. s. používané automatizované analyzátory močového sedimentu, pracujúce na princípe digitalizácie obrazu (snímaného mikroskopom a digitalizovaného kamerou s vysokým rozlíšením). Vyhodnotenie snímok získaných analýzou 3 mL vzorky je riadené softvérom, ktorý objekty na snímkach zatriedi na základe veľkosti, tvaru, kontrastu a štruktúry do príslušných kategórií, ako sú červené a biele krvinky, epitelové bunky, baktérie a podobne. Vzhľadom na autonómny vyhodnocovací softvér môže pri identifikácii a zatriedení dôjsť k nezhode. Riešením je ďalšie posúdenie výsledkov školeným pracovníkom, špeciálne pri pozitívnych močových sedimentoch, ako aj pri chýbajúcej chemickej analýze močov. Výsledky chemickej analýzy moču diagnostickými prúžkami sú hodnotnou komplementárnou informáciou v záujme objektívneho posúdenia močového sedimentu, najmä pri revízii nejednoznačných nálezov. Avšak chemická analýza diagnostickými prúžkami v dôsledku obmedzenej špecificity testov môže poskytovať výsledky ovplyvnené interferenciami, ako napríklad kyseliny askorbovej s dôkazom na prítomnosť hemoglobínu z červených krviniek. Z vyššie uvedených dôvodov automatizovaná analýza umožňuje dosahovať presné a správne výsledky potrebné na diagnostikovanie stavu pacienta aj v prípade základného vyšetrenia moču.

Kľúčové slová: močový sediment, automatizovaná analýza moču, manuálna mikroskopia, interferencie Iris iQ200

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Introduction

Chemical testing of urine samples (reagent strip method), identification and counting of particles is performed routinely to identify and monitor diseases of the kidney and urinary tract (1). Manual microscopy is extremely time consuming and has poor precision owing to variations in sediment preparation and counting technique (2, 3, 4, 5). Furthermore, visual urine microscopy is notoriously subjective (6). Improvements in automated systems are needed to eliminate labor intensive manual microscopy (7) given the large number of samples in clinical laboratories. Systems currently available to automate manual microscopy are based either on flow cytometry (8) or image-based microscopy analysis (9). Laboratories of Medirex company use automated urine microscopy analyzers iQ200 (Iris Diagnostics, Chastworth, CA) (9).

iQ200 analyzer

The iQ200 analyzer uses flow-imaging microscopy provided by high-definition camera and strobe lamp that stops the fluid motion in order to capture the images and detect particles. An Auto-Particle Recognition (APR) software works as trained neural network system for processing of images. APR software enables to sort particles according to size, shape, contrast and texture and thus to classify and quantify cells and formed particles in native uncentrifuged urine. Cells and particles can be sorted into categories and quantified as in table 1. Work station provides the viewing of the images when needed even though the sample has been already discarded.

Precision control is performed daily in the morning and calibration every 28 days. Before the testing of patients, iQ Focus, iQ Negative and iQ Positive (glutaraldehyde-preserved human red blood cells) control samples (Iris diagnostics) are run according to manufacturer's instructions. The iQ200 requires a minimum of 3 mL of urine for testing and quantifies particles per 2 µL of sample.

Precision study

Wah et al. (10) have published the evaluation of the analytic performance of the iQ200 based on a comparison with results from manual microscopy.

For the study of iQ200 within-run imprecision, 166 urine samples were analyzed a total of 20 times during the same day for red blood cells (RBCs), white blood cells (WBCs) and squamous epithelial cells (ECs). Under such conditions, 10 % of the samples tested had cell counts outside the 95 % confidence interval of the regression lines. The correlation study revealed discrepancies due to misclassification, disintegration of cells, abnormal morphological features (ghost and dysmorphic RBCs) or trapping of cells in mucus strands.

Sample assessment

It is necessary to emphasize that the precision study by Wah et al. (10) was performed via analyzer set-up as a walk-away system. In order to achieve the highest diagnostic accuracy and to prevent incorrect automatic classification of cells and particles at the laboratories of the Medirex company, the walk-away capability of the iQ200 is implemented only for samples possessing negative sediment and chemical analysis. After each assessment, analyzer flags samples that need to be reviewed by trained laboratory technician in case of absent chemical analysis or positive results of selected specimen. Review flags are assigned according to cut off values that convert quantitative data provided by the iQ200 analyzer into semiquantitative ranges correlating the image-based microscopy with qualitative chemical analysis (table 1). Setting the accurate cut off values for a lab's specific population is essential so there wouldn't be marked way too many flags or missed clinically important findings.

General approach for image reviewing and cell counts editing often includes comparison of urine sediment and chemical analysis. Chemical analysis based on dry-reagent strip is performed via on-line connection of analyzer Aution Max AX-4030 (Arkray, Japan) to the iQ200. Chemical analysis provides relevant supplementary information for assessment of cells and particles especially when misclassification or disintegration of cells occurs.

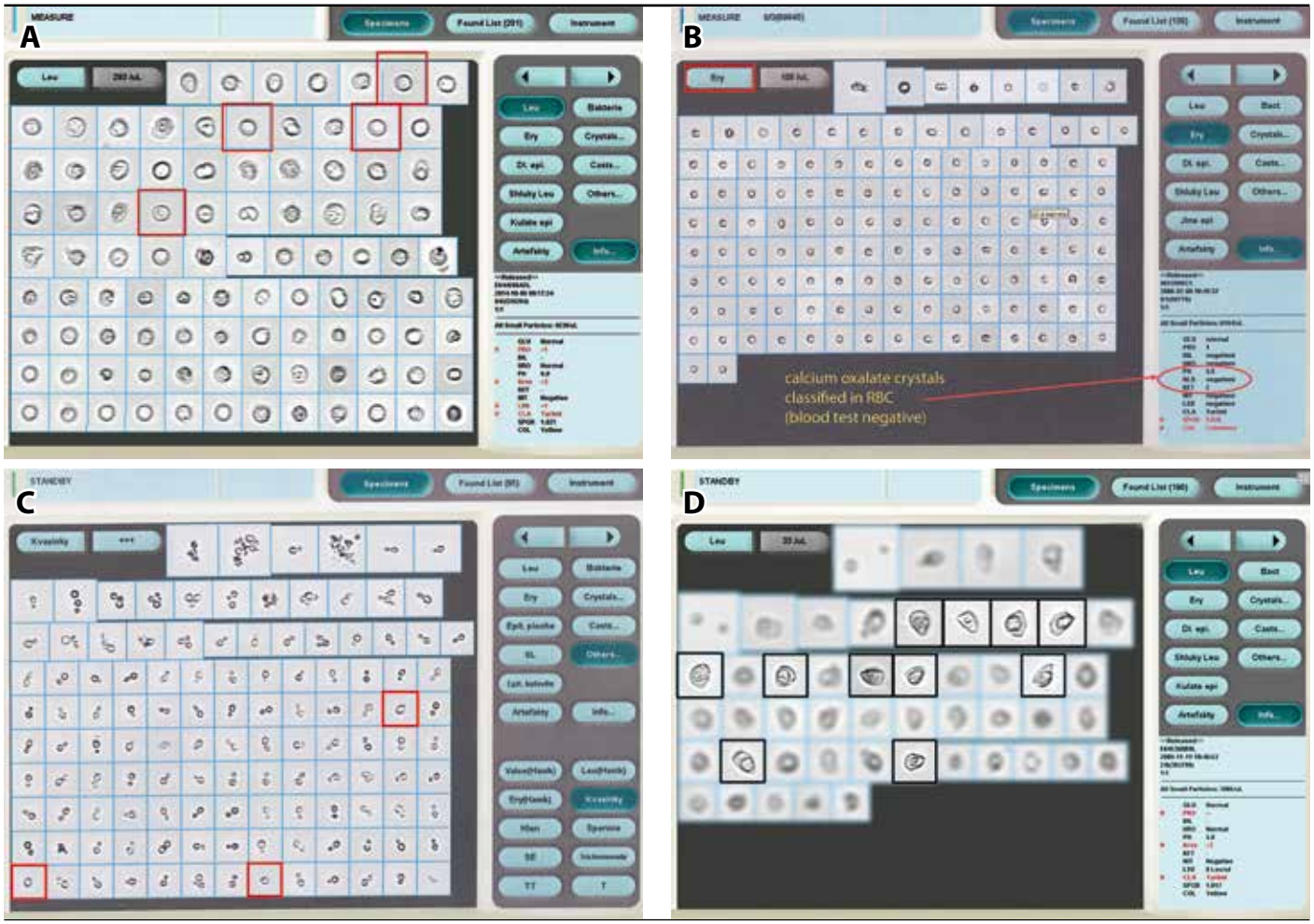
The most often misclassification of specimens occurs due to size and shape similarity as a consequence of morphological changes or high counts of particles. RBCs or/and WBCs are each other often misclassified when macroscopic hematuria or a pus is present (figure 1 A). Furthermore, oval calcium oxalate crystals (figure 1 B) or single-cell yeast (figure 1 C) can be added to RBCs. On the other hand, class of dysmorphic RBCs (especially acantocytes) can resemble to budding yeast, what subsequently requires the verification with manual microscopy. Renal tubular epithelial cells (figure 1 D) are rare findings that can be incorrectly sorted into WBCs clump. Often present mucus strands are prone to be classified as hyaline casts. On the contrary, APR software provides better identification of pathologic casts than manual counting. Translucent hyaline casts can be often missed by manual counting due to the use of bright-field microscopy. In order to prevent an overestimation of hyaline casts, manufacturer recommends verification of all casts by manual review of stored images. In general, the quality of stored images is sufficient for subclassification of casts with exception of cellular casts, in which the cellular detail is not always adequate for differentiation into cell specific subcategories. Small numbers of pathologic casts are often accompanied with higher protein excretion (more than 1+) what can refer to renal failure before it is manifested with increased serum creatinine level.

Table 1. Correlation of quantitative values obtained by iQ200 with qualitative values from chemistry analyzer. In case of absenting chemical test for particular category (marked as „none") an indirect reference is listed in corresponding row (marked as „ref.")

Category	Scale	
	Sediment analysis (particle count/μL)	Chemical analysis
RBCs	0 – 10	Negative
	11– 25	Traces
	26 – 80	1+
	81– 200	2+
	> 200	3+
WBCs	0 – 15	Negative
	16 – 70	Traces
	71 – 125	1+
	126 – 500	2+
	> 500	3+
WBCs clumps	Negative	None
	Rare	Ref. – positive WBCs
	Many	
Bacteria	Negative	Negative
	Rare	Positive
	Many	Strongly positive
Yeast	Negative	None
	Rare	
	Many	
Fungi	Negative	None
	Rare	
	Many	
Mucus	Negative	None
	Rare	
	Many	
Squamous epithelial cells	Negative	None
	Positive	
Transitional/Renal tubular epithelial cells	Negative	None
	Positive	Ref. – positive proteins
Hyaline casts	Negative	None
	Positive	
Cellular/Waxy casts	Negative	None
	Positive	Ref. – positive proteins
Crystals	None	None
	Present	Ref. – pH
Amorphous	Negative	None
	Rare	Ref. – pH
	Many	

Information about urine chemical properties such as pH, specific gravity, nitrites or ascorbic acid concentration can help in classification of the specimen when morphological features had changed. High count of bacteria (gram-negative bacteria are manifested as positive nitrites) often leads to increased pH of urine and thus to disintegration and abnormally shaped cells. Besides, low osmolality (low specific gravity) of urine can change morphology of RBCs subsequently sorted among WBCs. At the same time, chemical dry reagent strip is very prone to interference. Among all, ascorbic acid is the cause of the most important interferences in urine. It is able induce false negative result predominantly for RBCs (hemoglobin) and at higher concentrations for bilirubine, urobilinogen, bacteria (nitrites),

Figure 1. Morphological similarities of particles characterized in urine sediment: (A) misclassified RBCs (in a red box) among WBCs due to high counts of cells, (B) calcium oxalate crystals misclassified among RBC, (C) single cell yeast (in the red box) among budding yeast resembling to RBCs, (D) renal tubular epithelial cells (in the red box) sorted in the WBCs



WBCs (leukocyte esterase), etc. Furthermore, the very low specificity and sensitivity of dry-reagent strip for G-positive bacteria or lymfocytes connected to absent analysis of urine sediment can also provide false negative results. False positive sediment is another issue resulting from high counts of bacteria or squamous epithelial cells in improperly collected samples.

Conclusion

The number of urine samples received every day requires the use of automated analysis of urine sediment and chemistry. Such approach enables to achieve as objective, precise and accurate results as possible. Furthermore, trained technician can edit and review questionable findings when misclassification occurs. Taking into account such procedure, even fundamental laboratory examination of urine can help to diagnose the patient's condition fast and accurately.

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