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Gender's equality in evaluation of urine particles: Results of a multicenter study of the Italian Urinalysis Group ☆

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ABSTRACT

Objective: We performed a multicenter study to calculate the upper reference limits (URL) for urine particle quantification in mid-stream samples by using automated urine analyzers.
Design & methods: Two laboratories tested 283 subjects using a Sysmex UF-100, two other laboratories tested 313 subjects using Sysmex UF-1000i, whereas two other laboratories tested 267 subjects using Iris IQ®200.
Results: The URLs of UF-100 in females and males were 7.8/μL and 6.7/μL for epithelial cells (EC), 11.1/μL and 9.9/μL for red blood cells (RBC), 10.2/μL and 9.7/μL for white blood cells (WBC), and 0.85/μL and 0.87/μL for cylinders (CAST). The URLs of UF-1000i in females and males were 7.6/μL and 7.1/μL for EC, 12.2/μL and 11.1/μL for RBC, 11.9/μL and 11.7/μL for WBC, and 0.88/μL and 0.86/μL for CAST. The URLs of Iris IQ®200 in females and males were 7.8/μL and 6.6/μL for EC, 12.4/μL and 10.1/μL for RBC, 10.9/μL and 9.9/μL for WBC, and 1.1/μL and 1.0/μL for CAST.
Conclusion: The URLs obtained in this study were comparable to the lowest values previously reported in the literature. Moreover, no gender-related difference was observed, and analyzer-specific upper reference limits were very similar.

1. Introduction

The current reference approach for urine particle assessment entails microscopic examination by means of a cytometric chamber [1]. Nevertheless, this method requires trained operators and is time-consuming, so that it appears globally unsuitable for routine analysis of a large number of urine samples [2]. Standard microscopic examination of uncentrifuged urine usually lacks sensitivity, because the detection limit often approximates the upper limits of the reference range (URL) [3]. On the other hand, standard microscopic observation of urine sediment after centrifugation usually allows a correct morphological classification of the elements, although their quantification is still biased by a high degree of variability that can be mostly attributed to preanalytical causes (e.g., sample centrifugation and slide preparation), or analytical problems, especially high intra-observer variability [4]. Another source of bias is frequent reporting of results in a descriptive mode (i.e., rare, some, absent, etc.), rather than in terms of elements/microscopic field, as currently recommended [5].

The introduction of automated analyzers for quantification of urine particles has allowed a substantial standardization of urine particle analysis, due to the possibility of processing naive (uncentrifuged) samples, which has allowed to prevent most of the sources of preanalytical variability. In addition, these analyzers have virtually eliminated the wide intra-observer variability and have allowed generalized expression of test results in terms of elements/μL [6–8]. The improved analytical quality of data has also strengthened the need of reconsidering reference values, which appeared strongly influenced by sample collection procedure [1,9–11].

The Italian Urinalysis Group (GIAU) is composed of laboratory professionals operating in public and private laboratories broadly distributed throughout the national territory. The purpose of GIAU is to analyze all issues related to urinalysis, thus including publication of original articles and guidelines, organization of meetings of national relevance, along with local events and monothematic courses on urine microscopy

Abbreviations: CAST, CASTS/cylinders; CI 90%, Confidence Index 90%; EC, Squamous Epithelial Cells; IQR, Inter Quartile Range; RBC, Red Blood Cells/erythrocytes; URL, Upper Reference Limits; WBC, White Blood Cells/leukocytes.

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[8,12]. In a GIAU previously published study, a significant difference was found comparing two consecutive samples of the first morning micturition (i.e., the first from the first-void and the second from the mid-stream) for parameters such as white blood cells (WBC) and red blood cells (RBC), which are essential elements for assessment of renal and urinary tract disorders. Moreover, in this preliminary study on samples properly collected, we found that the reference values were lower than those previously reported in the literature, along with the lack of a significant gender-related difference in urine particle quantification [13]. Therefore, we planned a more extensive multicenter national study, to establish analyzer-specific reference limits for quantification of urine particles. As currently recommended by the International Society of Laboratory Hematology (ISLH) [14], the study included quantification of epithelial cells (EC), RBC, WBC and cylinders (CAST).

2. Materials and methods

The study was conducted according to the requirements of the Declaration of Helsinki, the protocol was approved by the Ethical review boards of the Team Leader (Medical Ethics Committees of the A-ULSS 17 Monselice), and written informed consent was obtained from all participants.

2.1. Sample collection

All samples collected between June and September 2012 from 863 healthy subjects (419 males and 444 females, age 18–70 years) were included in this study. All subjects were recruited from lab personnel, their relatives and blood donors. None of the study subject had clinically evident micturition disorders.

For each subject a sample from the first morning micturition was collected by using a clean catch mid-stream technique. Each subject received written instructions concerning sample collection, as recommended by recent guidelines [13]. The samples were collected in a 100 mL sterile disposable device and a 10 mL aliquot was then transferred in a vacuum tube, without preservative (Kima, Padova, Italy). All samples were rapidly transported to the laboratory, stored and evaluated within 2 h after collection. Each subject included in this study confirmed that collection had been performed as recommended and provided detailed indications about the time of delivery of the sample to the laboratory.

2.2. Analytical methods

Six Hospital-based clinical laboratories were included in this study, according to the prerequisites of using dip-stick automated analyzers for routine chemical urinalysis and automated analyzer for formed particle examination. The first two laboratories used a Sysmex UF-100 analyzer (Dasit, Cornaredo MI, Italy), and tested 283 subjects (147 males and 136 females), the second laboratories used a Sysmex UF-1000i analyzer (Dasit, Cornaredo MI, Italy), and tested 313 subjects (147 males and 166 females), whereas the final two laboratories used an Iris IQ®200 analyzer (Instrumentation Laboratories, MI, Italy), and tested 267 subjects (125 males and 142 females). A quantitative analysis was performed with automated analyzers for evaluation of urine particles, i.e., RBC, WBC, EC and CAST. In each Laboratory a full analyzer calibration was performed at the beginning of the study and thereafter with one month intervals. Quality control samples were run on daily basis, according to manufacturer's instructions.

2.3. Statistical analysis

By using specific software (MedCalc version 8.1.1.0, MedCalc Software, Mariakerke, Belgium) a parametric and non-parametric statistical approach was performed, by evaluation of mean and median with calculation of the 90% confidence interval (CI 90%). Analysis of data distribution was performed using Coefficient of Skewness, Coefficient of Kurtosis, and Kolmogorov–Smirnov test for Normal distribution. The URL was established at the 95th percentile, by using a method based on normal values and a non-parametric distribution percentile method [14]. A Mann–Whitney test for independent samples was also performed for comparison of data.

3. Results

The hypothesis of normal distribution was excluded after analysis of test results with Coefficient of Skewness, Coefficient of Kurtosis, and Kolmogorov–Smirnov test. It was hence decided to use a non-parametric approach for statistical evaluation, since this would be better suited for those circumstances where the lower reference limit of the parameter is clinically insignificant. Data dispersion was represented by using the Inter Quartile Range (IQR). The upper reference limits was established by considering 95th percentiles.

The values obtained with Sysmex UF-100 are shown in Table 1. This instrument also allowed quantification of bacteria-like particles (BLP), which values are also reported in Table 1. The values obtained with Sysmex UF-1000i are shown in Table 2. This instrument also allowed quantification of bacteria in a specific channel (BACT) and the BACT count is also reported in Table 2. The values obtained with IRIS IQ®200 are shown in Table 3.

When comparing the two laboratories that used the same analyzer, no significant differences were observed for EC, RBC, WBC, and CAST. Moreover, no significant differences were also found in quantification of BLP for laboratories that used Sysmex UF-100 and BACT for those using the Sysmex UF-1000i. Obviously, the values of BLP and BACT quantification differed significantly (data not shown). For any of the analyzers used no statistically significant differences were found for URLs of EC, RBC, WBC and CAST in relation to the gender of the study population. The results obtained with the three analyzers were also compared in order to verify whether common URLs could be identified and used

Table 1
Results obtained with Sysmex UF-100.

	EC/μL		RBC/μL		WBC/μL		CAST/μL	
	Females	Males	Females	Males	Females	Males	Females	Males
Sample size	136	147	136	147	136	147	136	147
Median	2.0	1.0	5.6	4.2	2.9	2.7	0.13	0.13
Inter Quartile Range	2.75	2.22	4.11	3.95	4.91	3.12	0.13	0.19
Skewness Coefficient	$p < 0.001$	$p < 0.001$	$p < 0.001$	$P < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Kurtosis Coefficient	$p < 0.05$	$p < 0.001$	$p = 0.05$	$P < 0.05$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Kolmogorov–Smirnov test	$p < 0.001$	$p < 0.001$	$P < 0.01$	$P < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$
Non-parametric statistic								
Upper limit 95th percentiles	7.8	6.7	11.1	9.9	10.2	9.7	0.85	0.87
90%CI	6.4–8.1	5.9–7.9	10.0–12.0	9.1–12.0	9.2–13.0	7.1–11.9	0.78–1.25	0.81–1.31
Mann Whitney test		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$

EC, epithelial cells; RBC, red blood cells; WBC, white blood cells; CAST, cylinders.