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UriSed — Preliminary reference intervals and optimal method for urine sediment analysis in newborns and infants

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ABSTRACT

Objectives: The aim of this study was to establish reference intervals for urine sediment in newborns and infants in the second month of life for the UriSed automated analyser and for bright field microscopy. We also aimed to provide an optimal protocol for UriSed analysis, which best corresponds to the results of manual microscopy.
Design and methods: Urine sediment analyses of 75 healthy newborns and infants in the second month of life were performed by manual microscopy and UriSed automated analyser (two modes: 15 and 20 images per sample). Images were then reviewed and manually corrected by an operator when needed.
Results: We observed statistically significant differences between bright-field microscopy and UriSed (when manual correction was not performed) for squamous epithelial cells and red blood cells counts ($P < 0.0001$). There were no differences based on the number of images per sample ($P > 0.05$). Upper reference values for bright-field microscopy and UriSed analyser taking 15 images per sample with manual correction (method we recommend) were as follows: squamous epithelial cells: microscope 8.7×10^6/l, UriSed 6.4×10^6/l, non-squamous epithelial cells: microscope 4.3×10^6/l, UriSed 3.9×10^6/l; erythrocytes: microscope 5.9×10^6/l, UriSed: 4.6×10^6/l; leukocytes: microscope 8.6×10^6/l, UriSed 9.9×10^6/l; hyaline casts: microscope 0×10^6/l, UriSed (no correction) 0.7×10^6/l.
Conclusions: We established preliminary reference intervals for urine sediment analysis in newborns and infants for UriSed and bright-field microscopy. We concluded that for routine laboratory examination of non-pathological urine it is enough to use the faster mode, with 15 images per sample, followed by a manual correction.

1. Introduction

After serum/plasma chemistry profiles and complete blood counts, urinalysis is the third most frequently used of the major diagnostic tests in clinical laboratories [1]. One part of a urinalysis is the urine sediment analysis, which is an uncomplicated, inexpensive test that can provide information that is useful for the diagnosis and prognosis of urinary tract diseases [2]. There is a wide range of techniques used for microscopic urine sediment analysis: contrast-phase or bright field microscopy; in standardised counting chambers or under a coverslip; with or without supravital staining and centrifuged or uncentrifuged samples of urine can be used. Although none of them provides both correct identification of different particles and their accurate quantities, the European Confederation of Laboratory Medicine (ECLM) recommended phase-contrast microscopy or supravital staining as methods that should be used for comparison and standardised visual microscopy under the coverslip as the reference method for urine sediment analysis [3].

Microscopic analysis of urine sediment is time consuming and is subject to significant variation due to the interindividual differences in skills and knowledge of those performing this analysis [4]. To overcome these problems, several automated systems for urine sediment analysis have become commercially available in recent years. Available automated analysers employ either digital image analysis (Iris iQ200 – Iris Diagnostics Inc., Chatsworth, California, USA;, UriSed – 77 Elektronika, Budapest, Hungary) or flow cytometry (UF-100 and UF-1000i – TOA Medical Electronics, Kobe, Japan). Automated analysers have significantly shortened the analysis time, thus leading to a reduced risk of cell lysis, bacterial contamination, or precipitation of salts when there is a prolonged time before testing [5–7]. Moreover, the reproducibility of results has been markedly improved. On the other hand, automated

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analysers may erroneously recognise urine sediment elements and manual correction may be required [5,8].

It has been pointed out that variable loss of urine sediment elements during centrifugation is among the major causes of error in the quantitative analysis of urine sediment [3,9]. Notably, automated analysers apply different protocols for sample centrifugation or they analyse uncentrifuged urine samples [10,11]. Thus, it is of great importance to establish distinct reference intervals for each automated method of urine sediment analysis. Furthermore, reference intervals should refer to population of those at a specified age [12]. There are few papers in the literature that aim to provide reference intervals for urine sediment elements in children and most those that exist focus on children older than one year of age [7,9,13–15]. Moreover, only reference intervals for manual microscopy and flow cytometry were established, while there are no reference intervals that could be used when urine sediment analysis is performed with systems employing digital image analyses [7,9,13–16].

As the reference intervals for urine sediment analyses for the youngest children were lacking, we aimed to establish reference intervals for urine sediment elements in newborns and infants up to two months of age using the UriSed analyser and bright field microscopy method for red blood cells, white blood cells, squamous epithelial cells, non-squamous epithelial cells and hyaline casts. It has been previously suggested, that an automated method is not as reliable as manual microscopy and we hypothesised there might be differences in the results of the urine sediment analysis of non-pathological urine samples, depending on the protocol used for UriSed analysis [8]. Accordingly, we aimed to provide an optimal protocol for UriSed analysis that best corresponds to the results of manual microscopy.

2. Design and methods

2.1. Specimens

The study was performed at the University Children's Hospital in Cracow, Poland. The Jagiellonian University Ethics Committee approved this study, which was designed in accordance with the principles of the Declaration of Helsinki. Hospitalisation procedures for each patient included in this study automatically contained routine urine sediment analysis; therefore, additional written consent of the parents was not necessary.

All urine samples, collected from children younger than 60 days, which were sent to the laboratory for routine urine sediment analysis during the years 2013–2015, were included in the study ($n = 1000$). Urine was collected into sterile bags that were placed over the labia or penis after the area around the urethra had been thoroughly washed. No preservatives were added to the urine samples. We followed ECLM guidelines [3] regarding the time from urine collection to laboratory analysis and the samples were examined within 1 h after collection. Both microscopic examination and the urine sediment analysis using the UriSed (in some countries known as SediMAX) automated analyser were performed on each sample. Subsequently, the medical record of each patient was evaluated. Only the children that were deemed healthy following physical examinations, clinical observations and results of laboratory tests (C-reactive protein, erythrocyte sedimentation rate, complete blood count, aminotransferases levels) and other tests performed during hospitalisation (including ultrasonography, computed tomography, magnetic resonance imaging) were included in this study. Most of these children were admitted to the hospital for observation when their behaviour seemed atypical to parents (especially young parents with a first baby), mainly lack of appetite and drowsiness, as well as when there was a suspicion of gastroaesophageal reflux or seizures. Finally, 75 children were accepted as a reference population (17 girls and 58 boys, all of them of Caucasian race, median age: 26 days (interquartile range: 12–41 days)).

We established upper reference intervals for the following urine sediment elements: squamous epithelial cells, non-squamous epithelial cells, red blood cells, white blood cells, and hyaline casts.

2.2. Visual microscopy

A medical laboratory technician performed a microscopic examination of the urine sediment using Vetriplast counting chambers (Vetriplast Roll, Arzergrande, Italy) according to the manufacturer's instructions with the aid of a bright field microscope (Hund Wetzlar H600, Wetzlar, Germany). The amount of native urine needed for the study was 5 ml. The samples were poured into centrifuge tubes with sediment bulbs (Equimed, Cracow, Poland) and centrifuged for 5 min at 1600 RPM (400 g). The supernatant was poured off and 0.5 ml of urine sediment remained in the bulb at the base of the tube. The urine sediment was mixed and placed into the Vetriplast chamber (total volume = 0.9 μl) to be counted. The elements were counted in 10 different small squares (volume of 10 small squares = 0.11 μl) To calculate the number of urine sediment elements per litre of urine, the following formula was used:

The number of urine sediment elements per litre of urine $= n \times 1/0.11 \times 10 \times 10^6$.

where:

n - the number of elements found in 10 small squares of the Vetriplast chamber;
1/0.11 - coefficient comprising the volume of 10 small squares of Vetriplast chamber;
10 - concentration factor
10^6 - coefficient to calculate the number of urine elements per litre;

2.3. UriSed

In the UriSed analyser, centrifuged samples of urine were automatically evaluated under a microscope connected to a camera. A total of 200 μl of urine was aspirated from a test tube and injected into a cuvette, after which a centrifugation step was performed (2000 RPM – 260 g, 10 s). This step is necessary to place all of the elements on one surface at the bottom of the cuvette, where the camera focuses. The cuvette was then placed in the microscope position and the camera took 5, 10, 15, or 20 images of separate fields of view in the centre of the cuvette using a built-in bright field microscope. The magnification is similar to that used for manual examination [17]. The images were then sent to the computer, which identified particular elements of urine sediment based on their structure, size, and contrast. Importantly, the images were shown on the screen and an operator could manually correct the automated results.

While analysing urine sediment samples with UriSed, UriSed software version 2.1.0.5 was used. Each sample was automatically analysed using two different modes: 15 images per sample and 20 images per sample; 20 images taken by the UriSed camera represented the examination of 3.2 μl native urine and 15 images represented the examination of 2.4 μl native urine [8]. Subsequently, a medical laboratory technician evaluated the images on the screen of the UriSed computer. Only one laboratory technician was involved in the urine sediment assessment for this study and he performed both microscopic examination and corrected UriSed results. The technician revised the automatic assignment of urine sediment elements if the UriSed had recognised them incorrectly or if the UriSed had not recognised some elements. Accordingly, we categorised the results into four different groups as follows:

1. 15 images per sample, with correction (group 15C)
2. 15 images per sample, no correction (group 15NC)
3. 20 images per sample, with correction (group 20C)
4. 20 images per sample, no correction (group 20 NC).

All results were expressed as the number of urine sediment elements per litre. The technician did not manually correct the numbers