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Coherent fluctuation nephelometry as a promising method for diagnosis of bacteriuria

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ABSTRACT

Objectives: Specialized analyzers are used to automate the diagnosis of bacteriuria in laboratory practice. They are based on analysis of microorganisms concentration in urine samples or recording the growth of urine microflora. Coherent fluctuation nephelometry (CFN) has high sensitivity and allows analyzing both parameters simultaneously. The aim of the study is to compare the effectiveness of CFN-based and flow cytometry based analyzers.

Design and methods: Total 117 urine samples from children were studied in parallel using the CFN-analyzer and UF-1000i (Sysmex), the results were confirmed by conventional microbiological methods.

Results: In 21 urine samples (18%), significant bacteriuria was determined (≥10^4 CFU/ml). The best diagnostic indicators were obtained while testing urine samples using the CFN-analyzer. The most efficient bacteriuria diagnosis is achieved by simultaneous analyses of microorganisms concentration in urine and growth of urine microflora (sensitivity – 95.2%, specificity – 96.9%, positive predictive value – 87%, negative predictive value – 98.9%, diagnostic odds ratio – 81.7, positive likelihood ratio – 30.5, negative likelihood ratio– 0.049, area under curve in ROC-analysis – 0.987). The CFN-analyzer allows the preliminary selection of negative urine samples, which do not require further analysis by conventional microbiological methods, thereby decreasing the number of cultures by 80.3%.

Conclusions: This study suggests that the CFN-analyzer is the effective tool for bacteriuria screening in children.

Introduction

Urinary tract infections (UTI) belong to the most common infectious diseases, making 20–49% of all nosocomial infections [1,2]. For correct UTI diagnosis, the knowledge about the presence of microorganisms in urine is needed. Such diagnosis is traditionally carried out by culture methods using solid nutrient media. New technologies have been introduced to laboratory practice for acceleration of diagnosis recently. Since most urine samples received for testing turn out to be negative, preliminary selection of

Abbreviations: AUC, area under curve; B, bacteriuria; CFN, coherent fluctuating nephelometry; CFU, colony forming units; G, growth time; T, turbidity; TN, traditional nephelometry; T & G, function of turbidity and growth time; UTI, urinary tract infections

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incoming samples allows to optimize the work process and to reduce the costs. A simple and rapid point-of-care dipstick bacteriuria screening test by determination of nitrites and leucocyte esterase levels in urine is developed [3], however its specificity and sensitivity are insufficient for use in laboratories. In veterinary practice, immunochromatographic test based on the detection of widespread bacterial pathogens in urine using specific antibodies begins to be used. Although the test cannot determine fungi and rare bacterial strains, it is promising for application in laboratory diagnosis [4], further multicenter studies are required to prove its diagnostic significance.

Flow cytometry based analyzers are used for bacteriuria screening in laboratory practice, for example UF-1000i (Sysmex). It combines cytofluorimetry and conductometry and can analyze the composition of urine sediment and also the concentration of microorganisms in urine. In case of significant bacteriuria, the concentration of microorganisms in urine sample is higher than in sterile samples and some of contaminated samples. That allows to define cut off level and to separate positive and negative samples prior to culturing to reduce the workload of the laboratory and to obtain negative results on the day of urine collection. UF-1000i was shown to be a useful tool for preliminary selection of urine sample for further bacteriuria testing [5]. Despite of high sensitivity, flow cytometry does not provide any information about viability of the detected microorganisms, which has negative influence on the specificity of the method.

Since 1980s nephelometry based systems, which detect scattered light intensity changes in time due to microorganisms division, are used for direct determination of viable microorganisms in urine samples [6]. Uro-Quick (Alifax) and BacterioScan 216Dx (BacterioScan Inc.) systems allow not only to perform bacteriuria screening, but also to test bacterial cultures for antibiotic susceptibility [7–10]. For bacteriuria screening, the analyzed parameter is growth delay time after start of incubation: in the case of significant bacteriuria, fast growth is observed, in the case of contamination, growth is delayed for a few hours. In nephelometry, high sensitivity is achieved only if cuvettes of high optical quality are used and complicated devices for stray light reduction are designed. It makes the used analyzers more complicated, while their sensitivity is still insufficient for turbidity measurement of urine samples in addition to recording growth curves. The data about urine turbidity would allow estimating the initial concentration of microorganisms, thus increasing diagnostic reliability of negative and positive urine sample determination.

Coherent fluctuation nephelometry (CFN) is a variant of nephelometry. In traditional nephelometry (TN), the mean intensity of scattered light in time is measured, while in CFN, the intensity fluctuations of scattered light are recorded. In TN, the stray light from optical parts of the device (first of all from the cuvette) and the light scattered by microorganisms are mixed and fundamentally cannot be separated in the resulting signal. In CFN, only particles moving in the cuvette make contributions to fluctuations of scattered light intensity, and the stray light from immovable parts of the device (cuvette, diaphragm, etc.) are subtracted and almost do not contribute to the resulting signal. Asymmetrical heating of the cuvette is used to provide the convection of the contained liquid, which makes the particles under investigation move with enough velocities. Furthermore, due to technological simplicity, in CFN light scattered to small angles (5–7°) can be detected easily, while in TN, stray light have the most impact at small angles. In turn, microorganisms also scatter light mostly to small angles, therefore recording intensity fluctuations of light scattered at low angles allows achieving the sensitivity limit of 600 CFU/ml in CFN, while the sensitivity of the best traditional one-angled nephelometers is limited by 25,000 CFU/ml [11].

High sensitivity of CFN allows not only to record growth curves of microorganisms at low concentrations, but also to estimate the concentration of microorganisms in urine samples by its turbidity. CFN-analyzers were used for bacteriuria screening by growth curve and by concentration of microorganisms separately [12] and also for antibiotic susceptibility testing [13].

The aim of the work is evaluation of diagnostic effectiveness of CFN-analyzer for bacteriuria screening.

Materials and methods

Total 117 urine samples were collected for routine microbiological testing in hospital and outpatient departments of the Federal State Autonomous Institution "Scientific Center of Children's Health" of the Ministry of Health of the Russian Federation (Moscow). Samples were stored in sterile containers without preservative agents at room temperature and analyzed within 2 h after collection. Every urine sample was divided into 3 parts. One part was used for culturing on *Uriselect* chromogenic agar (Bio-Rad, France) with the Ø4 mm calibrated loop for 24 or 48 h. The second part was tested using the CFN-analyzer, the third part was analyzed using UF-1000i.

The turbidity of the whole urine sample is analyzed using the CFN-analyzer, but not single cells flowing along the capillary, as in flow cytometry. For that reason, urine samples were centrifuged for 60 s at 3000 rpm (1700 g) to sediment large impurities (such as cells, salts, mucus). During such centrifugation, microorganisms do not sediment onto the bottom of the test tube and stay in the volume of the liquid. Then 0.5 ml of the supernatant was mixed with 0.5 ml sugar broth, placed into disposable 1 ml semi-micro cuvettes, and closed with disposable stoppers (LP ITALIANA SPA, Italy). The cuvettes were placed into CFN-P-12 analyzer (Medtechnopark Ltd., Russian Federation, Moscow) and incubated for 8 h. Muller-Hinton broth (Bio-Rad, France) with addition of 0.5% glucose was used in this work.

The results obtained using UF-1000i and CFN-analyzer were compared with those of culturing on solid media, which is considered the gold standard. The result of analysis of initial turbidity and growth curves using the CFN-analyzer were interpreted both separately and together. The objective of use of both analyzers is detecting and excluding from further testing maximal percentage of negative samples, and saving maximal percentage of positive samples for further testing by culturing on solid media.

Samples with positive cultures (N_+) tested with the analyzer can be either positive (true positive – N_{TP}) or negative (false negative – N_{FN}), $N_+ = N_{TP} + N_{FN}$. Samples with negative cultures (N_-) tested with the analyzer can be either positive (false positive – N_{FP}) or negative (true negative – N_{TN}), $N_- = N_{TN} + N_{FP}$. To estimate the diagnostic informative value of the test, the following indicators were used (Eqs. 1–9): sensitivity (the percentage of detected positive samples); specificity (the percentage of detected negative