Effect of the centrifugal force for preparing urinary sediment in the patients with urinary tract infection on the number of leucocytes and bacteria

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The urinary sediment microscopy are performed to predict various renal and urological diseases by observing erythrocytes, leucocytes, epithelium cells, casts, bacteria, fungi, protozoa, crystals.

In accordance with "urinary sediment examination procedure 2000" edited by the Japanese clinical laboratory standard meeting, it is provided that urinary sediment microscopy have performed using sediment of 10 mL urine after centrifuging of 500 ×g × 5 min. This centrifugal force is not enough for the bacterial collection.

There is no defined method to prepare urinary sediment when we perform gram-stain microscopy to detect bacteria, fungi, and leucocytes.

When we perform gram-stain microscopic test, we examined the suitable centrifugal force at the time of the preparation of the urinary sediments to detect 10^3 cfu/mL which was the number of the meaningful bacteria of the uncomplicated urinary tract infection.

Because it is necessary to observe bacteria, fungi and leucocytes in the examination of urinary tract infection, centrifugal force in the urinary sediment preparation is able to set higher speed and longer time than the normal urinary sediment preparation.

We evaluated suitable centrifugal force by counting bacteria in the supernatant after centrifuging at various centrifugal forces. The bacterial counting were performed with culture method and UF-1000i (Sysmex Corporation) that is flow cytometer.

It has been shown in this study that the centrifugal force of 500 ×g × 5 min was insufficient to collect bacteria, and the centrifugal force of 1,400 ×g × 10 min was at least necessary.

Key Words Urinary Sediment, Gram-stain, Centrifugal Force, Urinary Tract Infection, Bacteriological Examination

INTRODUCTION

Urinary sediment microscopy is performed to observe erythrocytes, leucocytes, epithelial cells, casts, bacteria, fungi, protozoa and crystals present in the urine in order to detect renal and urological diseases. Such testing is done when the urine is found to be positive in a dipstick test and when there is suspicion of urinary tract infection (UTI). Examination of Urine Sediment 2000 [1)] compiled by the Japanese Association of Medical Technologists stipulates sampling of 10 mL of mid-stream urine into a tube, and centrifuging at 500 ×g × 5 min to obtain 0.2 mL of residue. An 18 mm × 18 mm cover glass is then placed over 15 µL of residue on the glass slide and observed through a microscope at view number of eye piece with 20 and magnification × 400. When UTI is strongly suspected, mid-stream urine or catheter urine is collected in a sterile container for bacteriological tests, apart from the usual urinary sediment microscopy. The specimens are Gram stained and observed microscopically in a bacteriology laboratory. No standard procedure has been laid down for preparing urine sediments for such observation. We believe that 10 µL of the sample is usually used, taking into account the time required for drying. The magnification must be at least × 400 for detecting bacteria. Usually × 1000 is used. ***Table 1-a*** shows field of view-related data of an optical microscope. ***Table 1-b*** shows the theoretical bacterial counts per field when 10 µL of sediment is applied to half the area of a cover glass. From left to right, it gives bacterial counts per field for 3 different conditions: 1) × 10 eyepiece with the view number 20 and × 40 objective; 2) × 10 eyepiece with the view number 20 and × 100 objective; and 3) × 10 eyepiece with the view number 22

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and × 100 objective. For a 10 µL smear of non-centrifuged urine with a bacterial count 10^3 cfu/mL, which is considered the significant level of bacteria for diagnosing uncomplicated UTI, we can see only 1 bacterium in 100 fields at × 400 and 1 in 500 fields at × 1000. However a sediment is theoretically concentrated 50-fold, which makes the bacteria practically observable. For this reason, it is considered that microscopy of urine sediments is required for detecting the count of 10^3 cfu/mL, considered significant for uncomplicated UTI.
Based on this background, we investigated bacterial counts under different conditions of centrifugation in order to identify the appropriate centrifugal force and centrifugation time (min.) for preparing specimens for bacteriological examination.

MATERIALS AND METHODS

Five urine specimens A to E from individual UTI suspected patients were used in this study. With each specimen, 1 mL was dispensed into each of 5 microcentrifuge tubes. The non-centrifuged urine, and the supernatant after centrifuging at 500 ×g × 5 min, 1,400 ×g × 10 min, 3,000 ×g × 10 min, and 10,000 ×g × 5 min were analyzed using a Urine Particle Analyzer UF-1000*i* (UF-1000*i* ; Sysmex Corporation) for leucocyte, erythrocyte and bacteria counts. Concurrently, the urine specimens were cultured on blood agar medium (Kyokuto Pharmaceutical Industrial Co., Ltd.) and the bacterial counts determined.

Table 1-a *Field of view of an optical microscope*

View number of the eyepiece (× 10)	20	20	22
Magnification of the objective	40	100	100
Radius of the field (mm)	0.25	0.1	0.11
Area of the field (mm^2)	0.196	0.031	0.038
Area of cover glass (mm^2)	324	324	324
Fields in 1/2 the cover glass area	825	5157	4262

Table 1-b *Bacterial counts per field with different microscope configurations*

	Bacterial count per field		
	Microscope with view number 20	Microscope with view number 20	Microscope with view number 22
Bacterial count (CFU/mL)	10 × 40 magnification	10 × 100 magnification	10 × 100 magnification
Non-centrifuged urine			
10^3	0.012	0.002	0.002
10^4	0.121	0.019	0.023
10^5	1.21	0.19	0.23
Sediment			
10^3	0.61	0.10	0.12
10^4	6.06	0.97	1.17
10^5	60.60	9.70	11.73

RESULTS

Results for leucocytes and erythrocytes are respectively given in ***Fig. 1-a*** and ***Fig. 1-b***. 3 - 26 % of the leucocytes remained in the supernatant after centrifuging at 500 ×g × 5 min. This came down to 0 - 5 % at the centrifugal force of 1,400 ×g, and to less than 1 % at 3,000 ×g and 10,000 ×g. On the other hand, 7.7 - 44 % of the erythrocytes remained in the supernatant after centrifuging at 500 ×g × 5 min, some samples showing high counts in the supernatant. At 1,400 ×g and higher, the percentage came down to 0 - 12 %.

Fig. 2-a shows the bacterial counts determined by UF-1000*i* and ***Fig. 2-b*** those determined by the culturing method. Among the 5 specimens, E showed a bacterial count of only 10^2 cfu/mL or less by the culturing method even with non-centrifuged specimens. After centrifuging at 500 ×g × 5 min the supernatant was found to still contain 15 - 83 % of the bacteria when analyzed by UF-1000*i*. The results of bacterial culture method also showed that 21 - 82 % of the bacteria remained in the supernatant except in the one specimen (E) that the bacterial counts in the even if non-centrifuged urine had detected below the limit of detection. Specimens centrifuged at 1,400 ×g × 10 min showed 0 - 10 % of the bacteria remaining in the supernatant when analyzed using UF-1000*i* and also by the culturing method. At the higher centrifugal force of 3,000 ×g and 10,000 ×g, the supernatant was found to contain only 0 - 6 % of the bacteria by both the methods of analysis, except in the one specimen that had bacteria count below the limit of detection in the non-centrifuged state.

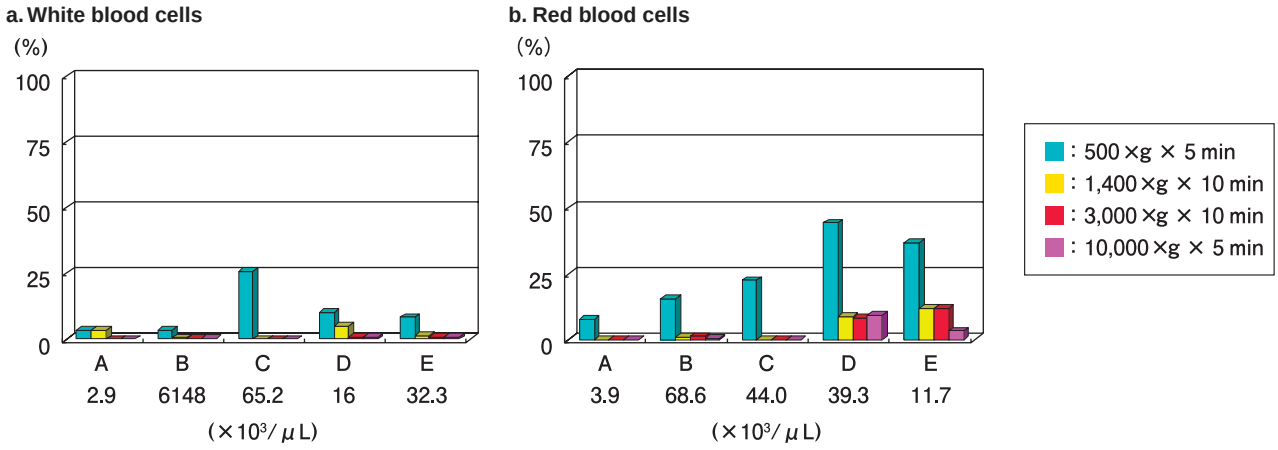

Fig. 1 *The percentages of erythrocytes and leucocytes in the supernatant after centrifuging, as determined by UF-1000i.*
(The count in a non-centrifuged sample was taken as 100 %).
The numbers given at the bottom are counts in non-centrifuged urine.

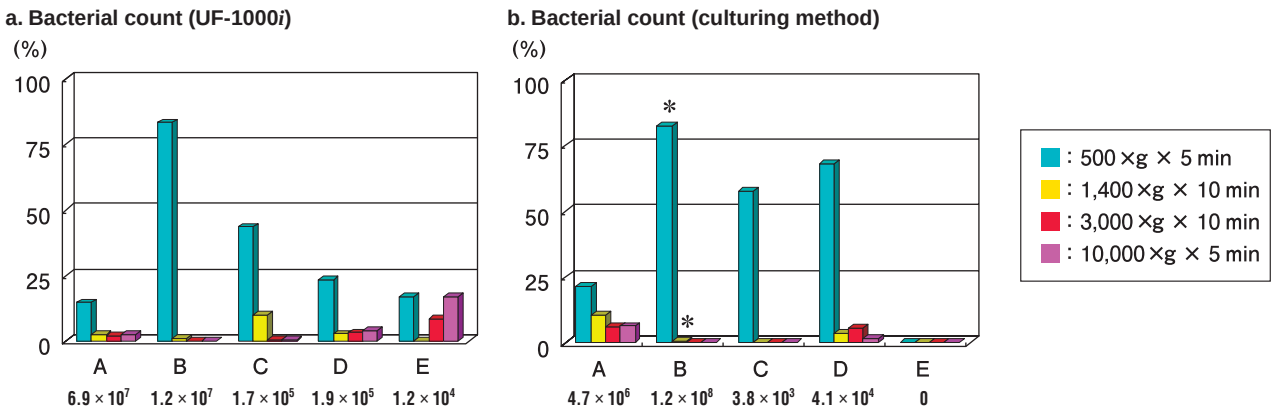

Fig. 2 *Percentages of bacteria remaining in the supernatant after centrifugation as determined by UF-1000i and the culturing method.*
(The count in the non-centrifuged sample was taken as 100 %).
The numbers given at the bottom are the counts in non-centrifuged urine.
**These are approximate values as the exact counts could not be obtained.*

DISCUSSION

Centrifugation at 500 ×g × 5 min is recommended for preparing urine sediment samples to avoid damage to crystals and casts. The American Society for Microbiology (ASM) recommends 400 ×g × 5 min [2]. Centrifugal force of this level is insufficient for proper collection of bacteria, and 3,000 ×g × 20 min is recommended for sedimentation of mycobacteria [3]. Apart from pyuria, bacteriuria also is to be checked for at the time of registering subjects for clinical trials of antimicrobial agents. Kawada et al. had reported that in microscopic examination of bacteriuria specimens after centrifugal sedimentation at 1,500 rpm × 10 min, the presence of at least one bacterial cell could be confirmed in each field when the bacterial count was 1×10^5 cfu/mL [4]. The review by Jenkins et al. has mentioned that in bacteriuria specimens of 1×10^5 cfu/mL or higher centrifuged at 2,500 - 3,000 rpm × 5 min, the sensitivity was 93 - 97 % and specificity not more than 88 % when examined unstained at × 400 magnification with the clinical significant cut-off settled at 1 or more bacteria per field. The sensitivity and specificity were respectively 98 % and 89 % for stained samples examined at × 1000 magnification [5]. In centrifuges with rotor radius of 15 cm, which are commonly used in Japan, 3,000 rpm corresponds to 1,500 ×g. The ASM manual also mentions that at least 1 bacterium can be seen per field when the bacterial count is 1×10^5 cfu/mL [6].

In this study, there was a need to detect a level of 10^3 cfu/mL, the significant level for diagnosis of uncomplicated UTI, through bacteriological examination by microscope testing involving Gram staining of smears. Therefore, we examined the effect of the centrifugal force used for preparing the sediment. Bacteria, fungi, and leucocytes alone need to be examined in bacteriological section. Thus, centrifugation can be done at a higher speed and for a longer time than in ordinary urine sediment analysis. In this study, we measured the proportion of bacteria remaining in the supernatant rather than in the sediment after centrifuging, as a preliminary test for determining the optimum centrifuging conditions. We have verified that centrifuging at 1,400 ×g × 10 min has no adverse effect on the morphology of leucocytes, including the phagocytic ones. Moreover, we considered the correct bacterial counts in the sediments also need to be evaluated simultaneously. The centrifugation force of 1,400 ×g is easily achievable with the centrifuges commonly used in laboratories in Japan, and a centrifugation time of 10 minutes is also practically feasible.

The results of this study suggest that centrifuging at 500 ×g × 5 min is insufficient for proper harvesting of the bacteria, and that at least 1,400 ×g × 10 min is necessary.

References

1) *Editorial Committee on Examination of Urine Sediment, Japanese Association of Medical Technologists Ed. Examination of Urine Sediment 2000. Tokyo: 3. (Japanese).*
2) *CLSI. GP16-A3 : Urinalysis ; approved guideline. 3rd ed. Wayne : 2009. 52p.*
3) *Committee for Mycobacterial Examinations, Japanese Society for Tuberculosis. Examination of tubercle bacillus guidance 2007. Tokyo: Japan Anti-Tuberculosis Association; 182p. (Japanese).*
4) *Kawada Y et al. Criteria for evaluation of clinical effect of chemotherapeutics on urinary tract infections. I. Method for the examination of urinary sediment. The Japanese journal of urology. 1970; 61: 633-638. (Japanese).*
5) *Jenkins RD et al. Review of urine microscopy for bacteriuria. JAMA. 1986 ; 255 : 3397-3403.*
6) *Murray PR et al. Manual of clinical microbiology. 9th ed. Washington, D.C. : American society for Microbiology ; 2007. 323-325.*