Field Volume of Urine Sediment Test – Comparison of Theoretical Volume with Practical Volume –

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The guideline for hematuria diagnosis was disclosed in March 2006. It has been defined as diagnostic criteria for hematuria that 5 or more red blood cells/HPF(high-power field, × 400) in the urinary sediment sample under microscopy and/or 20 or more red blood cells/μL by using flow cytometry technique with non-centrifuged urine sample are detected.

One HPF of microscopy for urinary sediment is theoretically equivalent to 0.45μL of non-centrifuged urine sample. However, in fact, there is discrepancy between theory and practice for some reasons. In this study, we examined the variance using KOVA slide.

We counted the urinary formed elements of primitive urine and urinary sediment. The results showed the tendency for the small elements to have greater discrepancy than large elements between theory and practice. It might well suggest that the small elements are easy to remain in the supernatant and to be adsorbed to the tube wall through a centrifugal process.

In our results, one HPF of microscopy for urinary sediment was equivalent to 0.31μL for squamous epithelial cells, 0.20μL for white blood cells, and 0.20μL for red blood cells.

This result meets the proportion of 20RBC/μL of primitive urine to 5RBC/HPF under microscopy in the diagnostic criteria for hematuria.

Key Words Non-Centrifugal Urine, Urine Sediment, Field Volume, KOVA Slide, SEKISUI Plate

INTRODUCTION

Guidelines for diagnosis of hematuria have been proposed in March 2006 by the Working Group for the Creation of Hematuria Guidelines (Japan). In these guidelines, a red blood cell count of about 5/HPF (high power field, × 400) or more in microscopic analysis of urine sediment has been defined as hematuria [1]. For flow cytometry (FCM) with uncentrifuged urine, about 20 RBC/μL or more has been defined as hematuria. However, these criteria are contradictory to the generally accepted assumption that one HPF field is equivalent to 0.45 μL of uncentrifuged urine. In the background of this difference is the fact that the actual filed volume in urine sediment analysis sometimes differs from the reported theoretical value [2]. We examined this difference using KOVA slides (Hycor Biomedical) and report the results here.

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MATERIALS AND METHODS

1. Specimens

52 patient urine specimens submitted to the Gifu Municipal Hospital for testing were used.

2. Materials

*1) KOVA slides (**Fig. 1**)*
These are slides called "KOVA® GLASSTIC® SLIDE 10 WITH GRIDS". They have cell counting grids and are used for analyzing urine sediment samples. Samples from 10 specimens can be prepared on one slide and viewed under the microscope. The count per µL can be calculated from the mean cell count per small grid. This allows quantification of urinary formed elements (particles) both in centrifuged and uncentrifuged urine samples [3,4].

*2) SEKISUI microscope plates (Sekisui Chemical, **Fig. 2**)*
These plates are made of glass, the slide and coverslip are integrated, and the fixed space between them enables the loading of a fixed volume of fluid. The various formed elements in the urine get uniformly distributed in the fluid. Three-window and five-window types are available, and the volume per window is 22.6 µL and 8.4 µL respectively in these two types. The five-window plates were used in the present study.

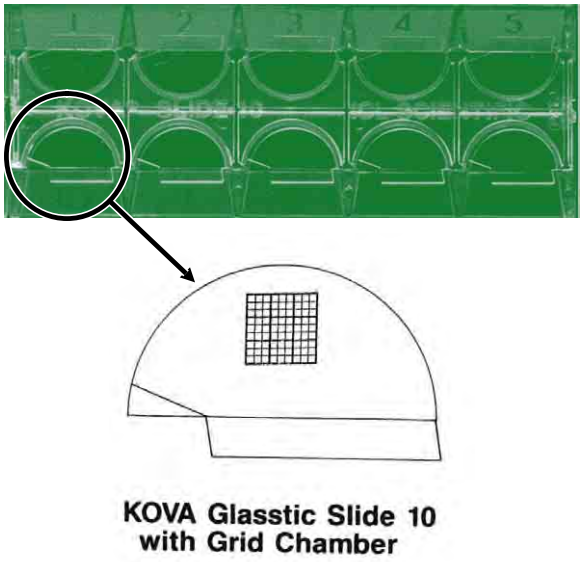

Fig. 1 *Coverslip part of the KOVA slide system*

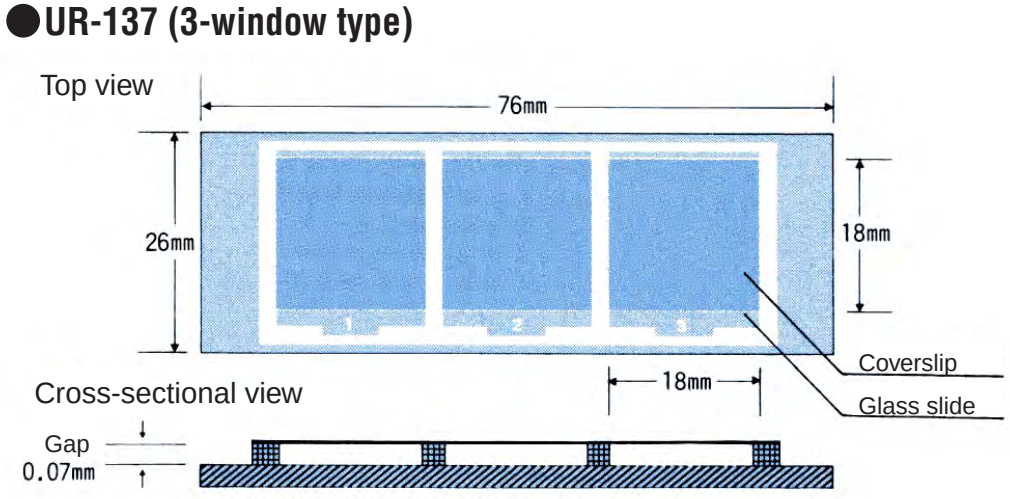

Fig. 2 *Coverslip part of SEKISUI microscope plate*

3. Methods

1) Uncentrifuged urine was loaded on a KOVA slide after thorough mixing, the formed elements were counted, and their number per µL determined (hereinafter referred to as "KOVA uncentrifuged").
2) Urine sediments were prepared according to the standard method (JCCLS) [5], and the formed element counts per µL of urine determined using KOVA slides and following the instruction manual of the slides (hereinafter "KOVA centrifuged")
3) Urine sediments were prepared according to the standard method (JCCLS), various formed elements were counted in 5 HPF fields on a glass slide, and their means calculated (hereinafter "Routine").
4) Urine sediments were prepared according to the standard method (JCCLS), various formed elements were counted in five HPF fields on a SEKISUI microscope plate, and their means calculated (hereinafter "SEKISUI").

4. Comparison of values

Table 1 shows the theoretical ratios of values determined by the different methods. The actual ratios of values measured by the different methods were compared with these theoretical ratios.

RESULTS

1. Comparison of KOVA centrifuged with KOVA uncentrifuged (*Table 2*)

As the theoretical ratio of KOVA centrifuged over KOVA uncentrifuged is 100%, the actual measured values should be the same. However, the actual KOVA centrifuged value was 52.7% of the actual KOVA uncentrifuged value for white blood cells, 47.8% for red blood cells (52.3% for isomorphic RBC and 35.1% for dysmorphic RBC), 108.6% for squamous epithelial cells, and 33.3% for hyaline casts. Thus, for all the urinary formed elements except for squamous epithelial cells, the centrifuged urine had lower counts than uncentrifuged samples.

2. Comparison of Routine with KOVA uncentrifuged (*Table 2*)

The actual Routine value was 20.1% of the actual KOVA uncentrifuged value for white blood cells, 19.7% for red blood cells (19.8% for isomorphic and 19.6% for dysmorphic RBC), 31.3% for squamous epithelial cells, and 88.7% for hyaline casts. The correlation between KOVA uncentrifuged and Routine values for all formed elements other than hyaline casts is shown in ***Fig. 3***. Overall there was good correlation, but the counts of the formed elements were considerably lower in Routine compared to KOVA uncentrifuged.

3. Comparison of SEKISUI with KOVA uncentrifuged (*Table 2*)

The actual SEKISUI value was 26.9% of the actual KOVA uncentrifuged value for white blood cells, 28.5% for red blood cells (30.8% for isomorphic and 10.2% for dysmorphic RBC), 51.5% for squamous epithelial cells, and 4.9% for hyaline casts. The ratios of white blood cells and red blood cells were relatively close.

Table 1 *Theoretical ratios of values determined by method Y over those determined by the method X*

		Y		
	Method	**KOVA centrifuged (/µL)**	**Routine (/HPF)**	**SEKISUI (/HPF)**
X	**KOVA uncentrifuged (/µL)**	**100%**	**45%**	**56%**
	KOVA centrifuged (/µL)		**45%**	**56%**
	Routine (/HPF)			**124%**

Table 2 *Actual ratios of values measured by method Y over those measured by the method X*

White blood cells

		Y		
	Method	**KOVA centrifuged (/μL)**	**Routine (/HPF)**	**SEKISUI (/HPF)**
X	**KOVA uncentrifuged (/μL)**	**52.7%**	**20.1%**	**26.9%**
	KOVA centrifuged (/μL)		**49.8%**	**54.7%**
	Routine (/HPF)			**131.9%**

Red blood cells

		Y		
	Method	**KOVA centrifuged (/μL)**	**Routine (/HPF)**	**SEKISUI (/HPF)**
X	**KOVA uncentrifuged (/μL)**	**47.8%**	**19.7%**	**28.5%**
	KOVA centrifuged (/μL)		**49.3%**	**61.9%**
	Routine (/HPF)			**174.2%**

Red blood cells (isomorphic)

		Y		
	Method	**KOVA centrifuged (/μL)**	**Routine (/HPF)**	**SEKISUI (/HPF)**
X	**KOVA uncentrifuged (/μL)**	**52.3%**	**19.8%**	**30.8%**
	KOVA centrifuged (/μL)		**44.0%**	**55.3%**
	Routine (/HPF)			**186.2%**

Red blood cells (dysmorphic)

		Y		
	Method	**KOVA centrifuged (/μL)**	**Routine (/HPF)**	**SEKISUI (/HPF)**
X	**KOVA uncentrifuged (/μL)**	**35.1%**	**19.6%**	**10.2%**
	KOVA centrifuged (/μL)		**64.5%**	**42.6%**
	Routine (/HPF)			**66.2%**

Squamous epithelial cells

		Y		
	Method	**KOVA centrifuged (/μL)**	**Routine (/HPF)**	**SEKISUI (/HPF)**
X	**KOVA uncentrifuged (/μL)**	**108.6%**	**31.3%**	**51.5%**
	KOVA centrifuged (/μL)		**41.9%**	**123.8%**
	Routine (/HPF)			**193.9%**

Hyaline casts

		Y		
	Method	**KOVA centrifuged (/μL)**	**Routine (/HPF)**	**SEKISUI (/HPF)**
X	**KOVA uncentrifuged (/μL)**	**33.3%**	**88.7%**	**4.9%**
	KOVA centrifuged (/μL)		**1165.6%**	**48.4%**
	Routine (/HPF)			**383.3%**

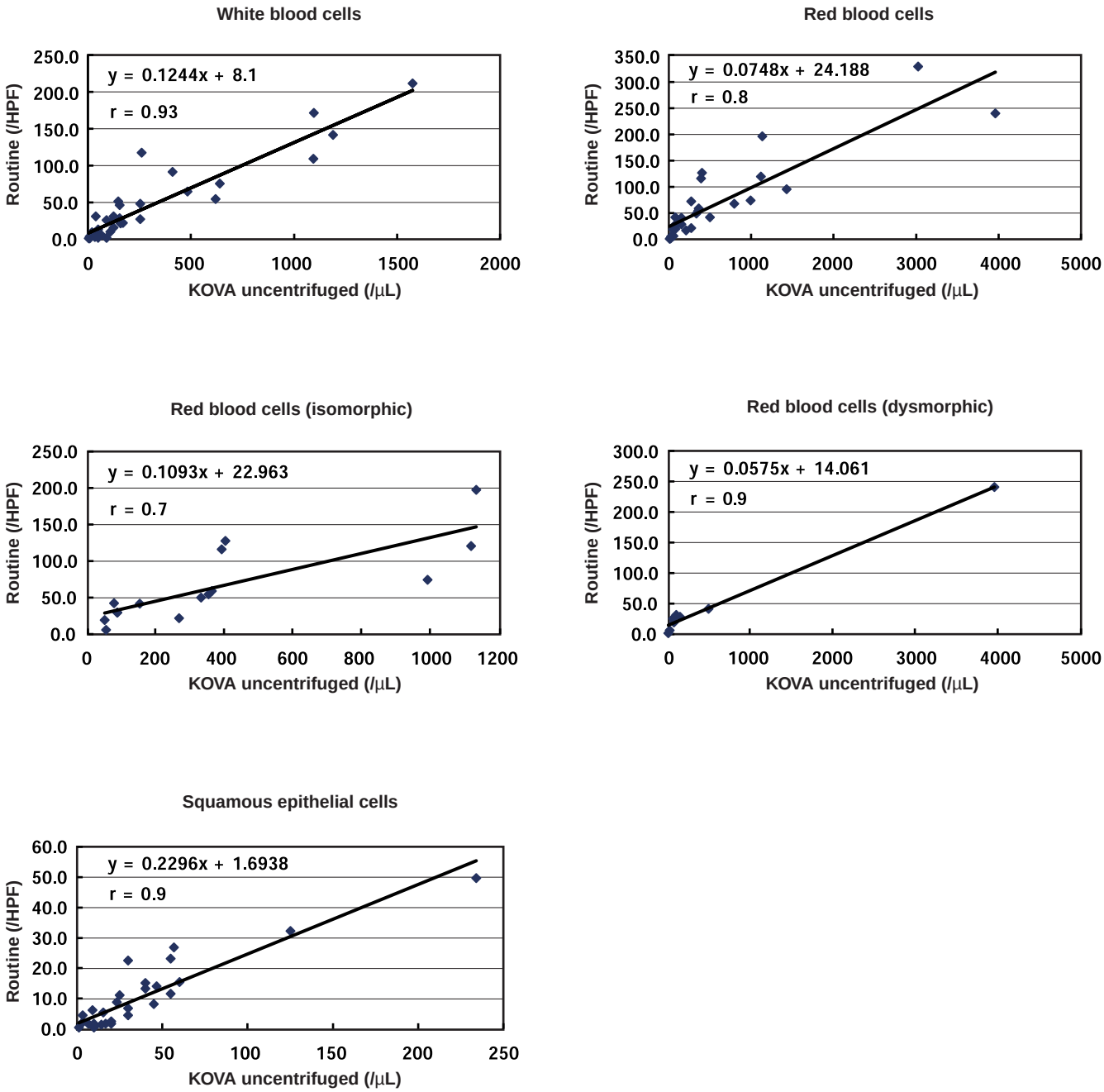

Fig. 3 *Comparison of actual values between KOVA uncentrifuged and Routine*

4. Comparison of Routine and SEKISUI with KOVA centrifuged (*Table 2*)

The actual Routine and SEKISUI values were respectively 49.8% and 54.7% of the actual KOVA centrifuged value for white blood cells, 49.3% and 61.9% for red blood cells (44.0% and 55.3% for isomorphic RBC and 64.5% and 42.6% for dysmorphic RBC), 41.9% and 123.8% for squamous epithelial cells, and 1165.6% and 48.4% for hyaline casts.

5. Comparison of SEKISUI with Routine (*Table 2*)

The actual SEKISUI value was 131.9% of the Routine value for white blood cells, 174.2% for red blood cells (186.2% for isomorphic and 66.2% for dysmorphic RBC), 193.9% for squamous epithelial cells, and 383.3% for hyaline casts.

DISCUSSION

Urine sediment analysis is an essential screening test for understanding urinary pathophysiology. The difficulty with this test is that all the procedures are done manually, which takes much time. In recent years, with advances in automation of urine sediment analysis through the use of flow cytometry, highly accurate counting of urinary formed elements at the level of number/μL in uncentrifuged urine sample has become possible. When comparing these measured values, it is considered that one HPF field in the JCCLS-recommended method of urine sediment analysis is theoretically equal to 0.45 μL of uncentrifuged urine. However, due to various reasons, there are considerable differences between the theoretical values and the actual measured values [2]. The present study also has yielded similar results.

The KOVA uncentrifuged and KOVA centrifuged should have theoretically yielded identical measured values. However, the KOVA centrifuged values were considerably lower except for squamous epithelial cells. This difference was greater with red blood cells than white blood cells. Among red blood cells, this difference was greater with dysmorphic RBC than isomorphic RBC. The reason for this could be that smaller particles are more likely to remain in the supernatant or get adsorbed on the tube walls during centrifuging. This can be guessed from the finding of a report by us in 2001 entitled "Examination by UF-100 of residual formed elements in the supernatant of centrifuged urine" that smaller the diameter of the urinary formed elements, the greater was their residual fraction in the supernatant [6]. Precipitation of urinary formed elements is affected by many parameters, as shown by Stoke's law. For instance, the particles get precipitated to a lesser extent when they have a smaller diameter, the difference in specific gravity between the fluid phase and the particle is smaller, the centrifugal force is smaller, and the viscosity of the fluid phase urine is larger [6, 7].

The theoretical ratio of the counts of formed elements measured by Routine over that measured by KOVA uncentrifuged is 45% in terms of count/HPF in uncentrifuged urine. But the actual ratio was 31.3% for squamous epithelial cells, 20.1% for white blood cells and 19.7% for red blood cells. Thus, there was almost a 2-fold difference between the theoretical and actual ratios and the factors discussed above are believed to be responsible for this difference.

In the comparison of SEKISUI with KOVA uncentrifuged, 1 HPF was taken as equivalent to 0.56 μL in terms of uncentrifuged urine. The actual ratios of all formed elements were smaller than the theoretical ratios. The count was particularly low for dysmorphic RBC, probably because of their small particle size. Moreover, with hyaline casts, the actual values were low because of the considerable maneuvering required for observing them as they were viewed in unstained specimens and the SEKISUI microscope plate thickness was 0.07 mm [8]. (This means some of the CASTs might be missed so then the count would be lower.)

CONCLUSION

Theoretically, 1 HPF in the standard method of urine sediment analysis is equivalent to 0.45 μL of uncentrifuged urine. However, the present study showed that the actually measured value varied depending on the formed elements present in the urine. The equivalent volume was 0.31 μL for squamous epithelial cells, 0.20 μL for white blood cells, and 0.20 μL for red blood cells. The value for red blood cells obtained here agreed with the equivalence relationship of 20 RBC/μL and 5 RBC/HPF (at magnification × 400) used in the diagnostic criteria for hematuria.

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