Comparison of Urine Analysis Using Manual and Sedimentation Methods

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ABSTRACT

Objective: *Microscopic examination of urine sediment is an essential part in the evaluation of renal and urinary tract diseases. Traditionally, urine sediments are assessed by microscopic examination of centrifuged urine. However, the current method used by the Georgetown Public Hospital Corporation Medical Laboratory involves uncentrifuged urine. To encourage high level of care, the results provided to the physician must be accurate and reliable for proper diagnosis. The aim of this study is to determine whether the centrifuge method is more clinically significant than the uncentrifuged method.*

Methods: *In this study, a comparison between the results obtained from centrifuged and uncentrifuged methods were performed. A total of 167 urine samples were randomly collected and analysed during the period April–May 2010 at the Medical Laboratory, Georgetown Public Hospital Corporation. The urine samples were first analysed microscopically by the uncentrifuged, and then by the centrifuged method. The results obtained from both methods were recorded in a log book. These results were then entered into a database created in Microsoft Excel, and analysed for differences and similarities using this application. Analysis was further done in SPSS software to compare the results using Pearson's correlation.*

Results: *When compared using Pearson's correlation coefficient analysis, both methods showed a good correlation between urinary sediments with the exception of white bloods cells. The centrifuged method had a slightly higher identification rate for all of the parameters.*

Conclusions: *There is substantial agreement between the centrifuged and uncentrifuged methods. However, the uncentrifuged method provides for a rapid turnaround time.*

Keywords: Certrifugation, High-power field, renal biopsy, urinary casts, urine microscopy

Comparación del Análisis de Orina Usando el Método Manual y el Método de Sedimentación

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RESUMEN

Objetivo: *El examen microscópico del sedimento de orina es una parte esencial en la evaluación de enfermedades renales y del tracto urinario. Tradicionalmente, los sedimentos de orina son evaluados mediante examen microscópico de orina centrifugada. Sin embargo, el método actual usado por el Laboratorio Médico de la Corporación del Hospital Público de Georgetown recurre a la orina no centrifugada. Con el propósito de estimular un alto nivel de cuidado, los resultados proporcionados al médico tienen que ser exactos y fiables para un diagnóstico apropiado. El objetivo de este estudio es determinar si el método de la centrifugación es clínicamente más significativo que el método sin centrifugación.*

Métodos: *En este estudio, se hace una comparación entre los resultados obtenidos a partir del método con centrifugado y sin centrifugado. Un total de 167 muestras de orina fueron recogidas aleatoriamente y analizadas durante el periodo de abril a mayo de 2010 en el Laboratorio Médico de la Corporación del Hospital Público de Georgetown. Las muestras de orina se analizaron primero microscópicamente por el método sin centrifugado, y entonces por el método con centrifugación. Los resultados obtenidos mediante ambos métodos fueron registrados en un en un diario de documentación. Estos resultados fueron entonces introducidos en un banco de datos creado en Microsoft Excel, y*

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analizados en cuanto a sus diferencias y similitudes usando esta aplicación. El análisis se realizó también más tarde mediante el software de SPSS para comparar los resultados usando la correlación de Pearson.

Resultados: *Al ser comparados mediante análisis basado en el coeficiente de correlación de Pearson, ambos métodos mostraron una buena correlación de los sedimentos urinarios, con excepción de los leucocitos. El método de la centrifugación tuvo una tasa de identificación ligeramente más alta para todos los parámetros.*

Conclusiones: *Existe una correspondencia sustancial entre los métodos con centrifugado y sin centrifugado. Sin embargo, el método que no emplea la centrifugación ofrece un tiempo de respuesta más rápido.*

Palabras claves: centrifugación, campo de alta resolución, biopsia renal, microscopia urinaria, cilindro urinario

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INTRODUCTION

The examination of urine, or uroscopy, is among the oldest tests in medicine, dating back to Babylonian physicians more than 6000 years ago. Today, even with the explosion in knowledge of renal disease and the accompanying sophistication of techniques to study these processes, 'simple' urinalysis remains the cornerstone for the evaluation of the kidney. It is the third major *in vitro* diagnostic screening test in clinical practice, only behind serum chemistry and complete blood count. Urinalysis is the first and most important laboratory test in evaluating a patient with suspected kidney disease. A correct urinalysis result offers a direct indication of the state of the patient's renal and genitourinary system and a monitor of other body systems. Traditionally, the complete examination of urine has been divided into a macroscopic and microscopic evaluation. The macroscopic analysis of urine includes assessment of its physical characteristics (appearance, odour, specific gravity) and chemical analysis. Microscopic analysis of the constituents of urine is performed on either an unspun (uncentrifuged) specimen or, more usually, the sediment from a centrifuged urine specimen (1–5).

To facilitate a high level of quality care, in both diagnosis and therapy, the results obtained from a microscopic urinalysis must be meaningful and the laboratory must encourage procedures that promote accurate recognition and diagnosis of disease state (6). Even though reliability and accuracy are demonstrated, there is still a lack of confidence in urine microscopic findings. This is dependent greatly on the inherent inaccuracies of the methods used (7). The manual uncentrifuged method had a significantly lower identification rate for all parameters of urinary sediments: casts, red blood cells (RBCs) and white blood cells [WBCs] (7).

Centrifugation of urine for microscopy is not done routinely in all countries. Centrifugation provides a concentrated specimen which helps to reduce the chance of missing important elements (7). A study was done by Pryles and Eliot to measure pyuria in centrifuged or uncentrifuged urine. The results distinctly provide a better correlation of bacteria with pyuria when the latter is measured in uncentrifuged urine (8).

Uncentrifuged urine microscopy is current practice in the Urology Department of the Georgetown Public Hospital Corporation (GPHC) Medical Laboratory. It requires less time for urgent urinalysis and also utilizes minimum laboratory resources. Since this analysis is not done with sediments, elements such as casts may be missed. In addition, the quantification of abnormal cells may be misleading to physicians. Therefore, this study seeks to evaluate results obtained from the centrifuged method and uncentrifuged method. This will guide management at the GPHC as to which method will provide information for the accurate diagnosis and treatment of patients.

METHODS

For the manual uncentrifuged method, a drop of well-mixed (using figure 8 motion) urine specimen was transferred using a pipette onto a microscopic slide and covered with a cover slip.

A sample of the same well-mixed urine (12 ml) was centrifuged in a centrifuge test tube at relatively low speed (2–3 000 rpm) for seven minutes until a moderately cohesive button was produced at the bottom of the tube. The supernatant was decanted and a volume of 0.2 to 0.5 ml remained inside the tube. The sediment was resuspended in the remaining supernatant by flicking the bottom of the tube several times. A drop of the resuspended sediment was transferred onto a glass slide for examination.

The sediment was first examined under low power to identify most crystals, casts, squamous cells and other large objects. Since the number of elements found in each field may vary considerably from one field to another, twelve fields were averaged. Next, examination was carried out at high power to identify crystals, cells and bacteria. The various types of cells were described as the number of each type found per average high power field (HPF) [*eg*: 1–5 WBC/HPF].

Ethical consideration

Ethical approval was granted by the Institutional Review Board of the Ministry of Health. Personal information of patients was not included in this research.

RESULTS

There was good agreement between the centrifuged and uncentrifuged methods when the results were compared except for WBCs and bacterial counts. The results in Table 1 show that the centrifuged method performed well at

Table 1: A comparison between the centrifuged and uncentriguged in the detection of PUS

	PUS Uncen	PUS Cen
NIL	62 (37.1%)	33 (19.8%)
+	34 (20.4%)	67 (40.1%)
2+	6 (3.6%)	19 (11.4%)
3+	3 (1.8)	6 (3.6%)
4+	1 (0.6%)	6 (3.6%)
5+	0	1 (0.6%)
1 in 12 Field	1 (0.6%)	0
2 in 12 Field	5 (3.0%)	0
3 in 12 Field	30 (18.0%)	4 (2.4%)
4 in 12 Field	5 (3.0%)	3 (1.8%)
5 in 12 Field	4 (2.4%)	2 (1.2%)
6 in 12 Field	10 (6.0%)	20 (12.0%)
7 in 12 Field	6 (3.6%)	3 (1.8%)
8 in 12 Field	0	3 (1.8%)
Total samples	**167**	**167**

detecting WBCs; only 19.8% of all the urine samples examined were found to contain no WBCs (Chi-square 435; $p < 0.05$). Whereas, with the uncentrifuged method WBCs were not detected in 37.1% of the urine samples resulting in a very high rate of false negative result. More WBCs +/HPF and 2+/HPF ranks were found in 40.1% and 11.4%, respectively with the centrifuged method when compared to 20.4% and 3.6% with the uncentrifuged method. In the centrifuged method, 12% of the urine samples contained six WBCs in 12 fields compared to 6% in the uncentrifuged. The values ob-tained for WBCs in the centrifuged method are almost twice that for the uncentrifuged method.

Bacterial count demonstrated significant difference (Pearson correlation 0.3, $p < 0.05$) between the two methods in Table 2. Considerably more samples, 47.9% and 28.8% contained 4+/HPF and 3+/HPF bacteria, respectively with the centrifuged compared to 11.4% and 7.2% with the uncentrifuged method. On the other hand, 56.3% and 25.1% of urine samples were found to have +/HPF and 2+/HPF bacteria, respectively, with the uncentrifuged compared to a lesser amount of 2.4% and 16.2% with the centrifuged method. Table 3 shows that a large percentage of the urine samples were found to contain no epithelial cells

Table 2: A comparison between the centrifuged and uncentrifuged in the detection of bacteria

	Bacteria Uncen	Bacteria Cen
NIL	0	8 (4.8%)
+	94 (56.3%)	4 (2.4%)
2+	42 (25.1%)	27 (16.2%)
3+	12 (7.2%)	48 (28.8%)
4+	19 (11.4%)	80 (47.9%)
Total samples	**167**	**167**

Table 3: A comparison between the centrifuged and uncentrifuged in the detection of epithelial cells

	Epithelial Cells Uncen	Epithelial Cells Cen
Nil	73 (43.7%)	41 (24.6%)
+	77 (46.1%)	83 (49.8%)
2+	0	32 (19.2%)
3+	0	4 (2.4%)
4+	0	1 (0.6%)
1 in 12 F	0	1 (0.6%)
2 in 12 F	1 (0.6%)	2 (1.2%)
3 in 12 F	6 (3.6%)	0
4 in 12 F	5 (3.0%)	0
5 in 12 F	1 (0.6%)	0
6 in 12 F	4 (2.4%)	2 (1.2%)
7 in 12 F	0	0
8 in 12 F	0	1 (0.6%)
Total sample	**167**	**167**

(43.7%) with the uncentrifuged method compared to 24.6% with the centrifuged (Pearson's correlation 0.065, $p > 0.05$). The percentages were close for the two methods in the +/HPF rank being 46.1% in the uncentrifuged and 49.8% in the centrifuged. However, in the 2+/HPF rank the centrifuged method performed best at 19.2% compared to 0.0% in the uncentrifuged method.

Table 4 shows RBC uncentrifuged to RBC centrifuged sample comparison. For the RBCs data, 19.2% of the urine sample contained 1+/HPF in the centrifuged compared to 10.8% in the uncentrifuged method. The centrifuged method also recorded higher values in the 2+/HPF and 6 in 12 fields/ HPF ranks being 6.6% and 6.0%, respectively, compared to 1.2% and 4.2 % in the uncentrifuged method ($p = 0.8$).

The predominant cast detected by both methods was hyaline casts (Table 5). Casts were detected in 12% of all the urine samples examined by the centrifuged method compared to 6.6% in the uncentrifuged method (Pearson's correlation 0.43, $p < 0.05$). For hyaline casts +/HPF and 2+/HPF rank, the centrifuged method measured 4.2% and 1.8% respectively, compared to 1.8% and 0.6% in the uncentrifuged method. The centrifuged method detected 0.6% of urine samples with six granular casts in 12 fields. The uncentrifuged method did not detect any pathological casts.

Table 4: A comparison between the centrifuged and uncentrifuged in the detection of RBCs

	RBC UnCen	RBC Cen
NIL	111 (66.5%)	97 (58.1%)
+	18 (10.8%)	32 (19.2%)
2+	2 (1.2%)	11 (6.6%)
3+	3 (1.8%)	2 (1.2%)
4+	1 (0.65)	3 (1.8%)
2 in 12 F	1 (0.6%)	0
3 in 12 F	9 (5.4%)	2 (1.2%)
4 in 12 F	13 (7.9%)	8 (4.8%)
5 in 12 F	1 (0.6%)	1 (0.6%)
6 in 12 F	7 (4.2%)	10 (6.0%)
7 in 12 F	0	0
8 in 12 F	1 (0.6%)	1 (0.6%)
Total sample	**167**	**167**

RBCs = red blood cells

Table 5: A comparison between the centrifuged and uncentrifuged in the detection of casts

	Cast Uncen	Cast Cen
NIL	156 (96.4%)	147 (88.0%)
H+	3 (1.8%)	7 (4.2%)
H2+	1 (0.6%)	3 (1.8%)
H3+	0	1 (0.6%)
H1 IN 12 F	4 (2.4%)	2 (1.2%)
H2 IN 12 F	1 (0.6%)	1 (0.6%)
H3 IN 12 F	2 (1.2%)	3 (1.8%)
AMU 4+	0	2 (1.2%)
GC 6 IN 12 F	0	1 (0.6%)
Total sample	**167**	**167**

H = hyaline; AMU = amorphous urate crystals; GC = granular casts

Of the 167 samples examined, 84.4% and 80.2% were negative for total crystals by both the uncentrifuged and centrifuged methods, respectively (Pearson's correlation 0.61, $p < 0.05$). The principal crystal found was amorphous urate crystals followed by calcium oxalate crystals (Table 6).

Other urinary sediments found were yeasts, spermatozoa and *T vaginalis* (Table 7). However, yeast cells predominated with 3.6% in the +/HPF rank for the uncentrifuged method in comparison to 1.2% in the centrifuged. *T vaginalis* were seen in 1.8% and 1.2% of the total urine sample by the uncentrifuged and centrifuged methods, respectively (Pearson's correlation 0.5, $p < 0.05$).

DISCUSSION

The study is the first of its kind done in Guyana. Limited studies were available to support this study since automated urinalysis systems are becoming more extensively used in developed countries. Reasons are based on the fact that

Table 6: A comparison between the centrifuged and uncentrifuged in the detection of crystals

	Crystal UnCen	Crystal Cen
NIL	141 (84.4%)	134 (80.2%)
AMU +	3 (1.8%)	0
AMU 2+	5 (3.0%)	2 (1.2%)
AMU 3+	2 (1.2%)	2 (1.2%)
AMU 4+	0	8 (4.8%)
AMP 4+	2 (1.2%)	1 (0.6%)
COA +	3 (1.8%)	3 (1.8%)
COA 2+	0	4 (2.4%)
COA 3+	2 (1.2%)	2 (0.6%)
COA 4+	0	1 (0.6%)
UA +	1 (0.6%)	1 (0.6%)
SUL +	1 (0.6%)	1 (0.6%)
SUL 4+	0	1 (0.6%)
TP 4+	0	1 (0.6%)
COA 1 IN 12 F	0	1 (0.6%)
COA 3 IN 12 F	2 (1.2%)	1 (0.6%)
COA 4 IN 12 F	1 (0.6%)	0
UA 1IN 12 F	1 (0.6%)	0
UA 3 IN 12 F	0	1 (0.6%)
UA 6 IN 12 F	1 (0.6%)	0
AMP 4+ and TP +	1 (0.6%)	0
AMP 4+ and TP3+	0	1 (0.6%)
UA 4+ and COA 6 in 12 F	0	1 (0.6%)
COA 2+ and AMU 2+	0	1 (0.6%)
COA + and AMU	1 (0.6%)	0
Total Sample	**167**	**167**

AMU = amorphous urate crystals; AMP = amorphous phosphate crystals; UA = uric acid crystals; COA = calcium oxalate crystals; SUL = sulphates; TP = triple phosphate crystals

Table 7: A comparison between the centrifuged and uncentrifuged in the detection of other urinary sediments

	Other Uncen	Other Cen
NIL	154 (92.2%)	150 (89.8%)
Y+	6 (3.6%)	2 (1.2%)
Y2+	0	2 (1.2%)
Y3+	0	1 (0.6%)
Y3 in 12 F	0	1 (0.6%)
Y4 in 12 F	1 (0.6%)	0
Few spermatozoa	1 (0.6%)	1 (0.6%)
Occasional spermatozoa	1 (0.6%)	1 (0.6%)
Many spermatozoa	0	1 (0.6%)
T vaginalis	3 (1.8%)	2 (1.2%)
Y+ and occasional spermatozoa	1 (0.6%)	0
Unable to identify	0	6 (3.6%)
Total Sample	**167**	**167**

Y = yeast

traditional microscopy of the urinary sediment is labour-intensive, time-consuming and imprecise and has wide variability (9). Attempts have been made to reduce the variation of manual analysis involving the use of uncentrifuged samples and automation of urinalysis. The automated urinalysis procedure can also save labour and time and is more feasible for the high volume laboratory workload (10, 11).

Performance of urine microscopy is vital to identify and monitor patients with diseases of the kidney and urinary tract, as well as metabolic, cholestatic and haemolytic diseases (12). In medical laboratories, urine microscopy is performed by the observation of urine sediments prepared by the centrifugation method rather than the uncentrifuged method. However, obtaining accurate and reliable results from this method may be prevented by methodological problems (13, 14). The current method of urine microscopy at the GPHC Medical Laboratory is the uncentrifugation method. A wide range of concentrations of cellular components were accurately detected by the two methods compared in this study. Although a difference was observed between the analytic results, it was not significant since both methods showed significant correlations for all of the variables measured except for WBCs. Moreover, the uncentrifuged urine microscopy had an insignificantly lower detection rate for most of the parameters measured. In spite of the good agreement between the two methods, the results were not identical since the centrifuged urine microscopy tends to detect greater values for WBCs and bacteria. Great difference was demonstrated between the two methods for bacterial count. However, the clinical value of any sediment bacterial count is questionable, since the routine manual counting of bacteria is a very crude and variable technique. The medical technologist may find it difficult to interpret small particles "cocci" as "bacteria" except the rod-like bacteria (15).

The centrifuged method tends to give positive results of casts more frequently when compared to the other method despite a significant correlation. Turbid urine samples should be diluted before running. In addition, reducing the error rate could be achieved by cross checking the results with dipstick data such as RBC *vs* blood, WBC *vs* leukocyte esterase, casts *vs* protein, bacteria *vs* nitrite.

The centrifuged method identified more epithelial cells than did the uncentrifuged. On the other hand, there was no significant quantitative difference between the two methods on detecting RBCs since both methods performed equally well. It has been recommended that quantitative urinalysis be performed using urine samples without centrifugation (16–18). The average time taken to prepare one urine sample by individual methods was assessed. The uncentrifuged method took approximately two minutes whereas the centrifuged required ten minutes. From the evaluation, the uncentrifuged method can perform urinalysis in a more time-saving manner than the centrifuged method since sediment preparation by centrifugation is absent.

CONCLUSION

This study found that both uncentrifuged and centrifuged methods displayed good correlations with each other in detecting most of the urine sediments observed, with the exception of WBC counts. Although the centrifuged method had a slightly higher identification rate for the other urine sediments examined, the results were comparable. Thus, when combined with urine chemistry analysis, both methods should provide valuable information as a screening tool in routine urine analysis. However, the uncentrifuged method will provide a rapid turnaround time for issuing results at GPHC Medical Laboratory.

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