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Effect of specimen placement time and temperature on RBC detection in urine

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Abstract: The accuracy of red blood cell count and morphology in urine samples is of great value for clinical diagnosis. In this study, a total of 215 urine samples were collected from affiliated Hospital of Hebei University from October 2022 to April 2023, among which 112 cases were placed at room temperature, 103 cases in 4℃ refrigerator, UF-5000 urine formed analyzer measured their placement after 0.5h, 1h, 2h, 4h, 6h, observed the changes of red blood cell morphology at each time point and analyzed the significant difference with 0.5h by t-test. At room temperature, red blood cells in urine at 2h, 4h and 6h were significantly compared with 0.5h (P <0.05), and at 1h and 0.5h were not significant (P> 0.05). In 4℃ environment, the results in urine at 4h and 6h were compared with those at 0.5h (P <0.05), and at 1h, 2h and 0.5h were not significant (P> 0.05). Therefore, urine samples at room temperature should be completed before 2h, preferably not more than 1h. Under 4℃ refrigerator storage conditions, the test can be completed before 4h, preferably no more than 2h.

1. Introduction

Urine formation subexamination is an important part of the routine urine examination items. At present, urine sediment analyzer is used at home and abroad to detect urine sediment, and the principles of electric impedance, light scattering and flow cytometry are made of fresh and clean urine specimens by flow cytometry[1]. In daily work, due to various reasons, the urine specimens cannot be submitted for inspection in time (mainly inpatients). Secondly, when the patient specimens increase the workload, the time interval of the specimens to the computer test is extended, resulting in the changes of red blood cells in the urine. The detection of red blood cells in urine specimens is an extremely important indicator of urine formation, which provides important clinical reference information for the diagnosis of urinary system diseases and the source of hematuria[2].

2. Materials and Methods

215 outpatient urine samples were collected from June 2022 to April 2023, ensuring the specimens were fresh and clean middle urine with characteristics of non-uniform microscopic hematuria, among which 112 cases were placed at room temperature, 103 cases in 4℃ refrigerator.

They were tested by UF-5000 urine sediment analyzer (Sysmex, Japan) after quality control at 0.5h, 1h, 2h, 4h, 6h respectively, obtaining red blood cell count and related parameters, and the changes of red blood cell dysplasia were observed by contrast microscope (Olympus CX-41, Japan). Artificial microscopic scopy: normal cells of urine samples showed double concave disc, with equal size, intact cell membrane and uniform hemoglobin content. Abnormal urine red blood cells have small volume, irregular size, and heterogeneous hemoglobin. There are common bread like cell membrane rupture, copper coin cells, unequal cells and red blood cell debris, cells have dental spines or serrated, shadow red blood cells, etc[3]. UF-5000 urine sediment analyzer with a urine volume of 0.18 microliters; 1 microliters of urine red cell count was about 5.57 times the average number of red blood cells per high microscopic field; the information (RBC-INF 0) 1.0 indicates that it is homogeneous in urine, 2.0 is inhomogeneous and 3.0 is mixed [4]. Statistical treatments were analyzed using the paired sample t-test in spss22.0 and P <0.05 showed statistical significance.

3. Results

3.1 The effect of urine placement time at room temperature on the red blood cell count results at room temperature.

P> 0.05 between 1h and 0.5h. P <0.05, as shown in Table 1;

Table 1: Relationship between the time of urine placement at room temperature and the red blood cell count

storage period	Red blood cell count ($\pm S)\bar{X}$	t price	*P*
0.5h	221.13±114.12	-	-
1h	221.09±114.10	0.469	>0.05
2h	199.61±113.98	14.666	<0.05★
4h	179.58±120.58	11.852	<0.05★
6h	171.54±119.94	14.206	<0.05★

Note: ★ indicates that the time group is compared with 0.5h.

3.2 Effect of 4℃ environmental urine placement time on RBC count results.

Compared with the urine red blood cell count at 0.5h, P> 0.05 at 1h and 2h was not statistically significant. However, P <0.05 was statistically significant as shown in Table 2.

Table 2: 24℃ Association between urine placement time and red blood cell count

storage period	Red blood cell count ($\pm S)\bar{X}$	t price	P
0.5h	141.00±79.39	-	-
1h	140.86±79.34	1.879	>0.05
2h	139.41±77.11	1.976	>0.05
4h	118.42±66.97	16.563	<0.05★
6h	111.27±64.03	18.183	<0.05★

Note: ★ indicates that the time group is compared with 0.5h.

3.3 The relationship between RBC morphology and urine placement time at room temperature is shown in Table 3.

At room temperature, the results of heterogeneous cells for 1h and 0.5h (P> 0.05), and the results of abnormal cells for 2h and 0.5h (P <0.05);

Table 3: Relationship between heterplastic erythrocytes and placement time at room temperature

storage period	Number of heteromorphic red blood cells▲($\bar{X}$ ±S)	t price	P
0.5h	27.41±12.29	-	-
1h	27.49±12.19	-1.349	>0.05
2h	30.86±13.38	-20.146	<0.05★
4h	32.46±13.82	-17.237	<0.05★
6h	34.07±14.03	-16.89	<0.05★

Note: ★ indicates P 0.05 when comparing this time group with 0.5h.▲ Number of heteromorphic red blood cells in 100 red blood cells in the specimen.

3.4 The relationship between RBC morphology and urine placement time at 4 ℃ is shown in Table 4.

There was no significant difference in the results of abnormal erythrocytes between 2h and 0.5h after urine placement (P>0.05), but there was significant difference between 4h and 0.5h after urine placement (P<0.05).

Table 4: The relationship between hypomorphic erythrocytes and placement time at 4℃

storage period	Number of heteromorphic red blood cells▲($\bar{X}$ ±S)	t price	P
0.5h	36.84±17.62	-	-
1h	36.77±17.60	0.940	>0.05
2h	36.84±17.48	0.004	>0.05
4h	43.00±17.60	-13.455	<0.05★
6h	45.69±18.10	-15.425	<0.05★

Note: ★ indicates P 0.05 when comparing this time group with 0.5h.▲ Number of heteromorphic red blood cells in 100 red blood cells in the specimen.

4. Discussion

The formation of urine is a complex process. First, the glomerular filtration process, plasma water, inorganic salt ions, and small molecule solutes are filtered into the glomerulus to form prouria, and then the reabsorption and secretion of the tubular collecting duct. And red blood cells in the urine, from the glomerulus before the body of vascular pressure, to the glomerulus by glomerular basement membrane, then by the tubules different PH, osmotic pressure, together with other components of urine may be because of the destruction of red blood cells, can make the red blood cells in the urine number and morphological changes. This experiment found that with the extension of placement time, the red blood cell count showed a gradual downward trend, in which the specimen with more bacterial count would affect the red blood cell count. Some studies showed that the reason why the number of bacteria affects the red blood cell is that the urine PH value is alkaline when bacteria multiply, and the PH may precipitate urine crystals and accelerate the dissolution of red blood cells; In addition, the large number of some cocci may lead to the false increase of red blood cell count. Ambient temperature is also critical for urine specimens. Increasing temperature may multiply bacteria in urine specimens. Therefore, preservatives are added to the 24h urine protein project specimen containers in summer to prevent the results. In this experiment, it was found that in addition to the number of bacteria would affect the red blood cell count, the crystallization of urine samples and the increase of yeast number also increased the red blood cell count in urine, which may be caused by the instrument putting the crystallization and yeast count into the red blood cell count[5]. Therefore, in order to ensure the single and accurate variables of the

experiment, attention should be paid to avoid the influence of bacterial quantity and crystallization quantity.

The detection of red blood cell morphology in urine specimens has important clinical implications for the source of hematuria. On the urine sediment analyzer, the red blood cell information message 1.0 is expressed as uniform nature, generally non-glomerular hematuria[6]; Report 2.0 is a non-uniform nature, mostly glomerular hematuria. The specimen contains more than two kinds of heteromorphic red blood cells, of which more than 80% are renal hematuria, while the proportion of heteromorphic erythroid cells is less than 50% is called non-renal hematuria[7-9]; At 3.0, it is expressed as a mixed hematuria specimen, that is, both normal and abnormal red blood cells appear and a relative proportion of 20%~80% mixed hematuria[10]. This experiment concluded that the proportion of heteromorphic erythrocytes in urine increased with the prolonged placement time. The reason may be that the changes such as osmolality and PH affected the morphology of urine, and the number of erythrocytes gradually increased with the loss of hemoglobin. In the experiment, the changes of red blood cell morphology and Hb loss in the urine at 4h and 6h tended to be in a stable state relative to the first four hours, and the changes between the two can be further studied in the future.

Hospital actual daily workload is very much, both outpatients and ward patients specimens, outpatients urine specimens retention and send more timely, specimen placement time and environment influence is relatively small, and ward urine specimens in the process of specimen retention and inspection time uncertainty is greater, so make the quality control before the experiment is the key to ensure the accuracy of the test results[11]. Through this experiment, it is found that under special circumstances (large main index), the test time of red blood cells at room temperature should be completed before 2h, preferably less than 1h. If the huge workload cannot be completed, it can be placed in the 4℃ refrigerator condition. At this time, the test should be completed before 4h, preferably no more than 2h. This is conducive to ensure that the results of each patient can be accurate and not delayed, and implement the lofty concept of "patient-centered".

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