Research Article

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Diagnostic pitfall of carryover: in automatic urine analyzers
İdrar otoanalizörlerinde bulaşmanın diagnostik tuzağı

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Abstract

Objective: We aimed to find out whether there is significant carryover effect which causes false-positive hematuria on red blood cells (RBCs) in automatic urine chemistry (DIRUI H-800) and sediment (DIRUI FUS-200) analyzers.

Methods: Twenty-four samples with gross hematuria selected as containing high RBC concentration and forty-eight samples which had both negative result in dipstick and 0/hpf in microscopic examination selected as containing low RBC concentration. Carryover% was calculated via the formula \[\text{carryover\%} = 100 \times \frac{(b_1 - b_2)}{(a_2 - b_2)}\]. Carryover effect within results was analyzed with Wilcoxon test.

Results: Carryover% was very high (67%) in urine chemistry analyzer. Carryover% of urine sediment analyzer was found 0.4% whilst false-positive hematuria percentage was 87.5% for the first samples came after gross hematuria and 6.6% for the second samples. The first samples analyzed after gross hematuria had significantly higher (p < 0.001) results than the second samples in both analyzers.

Conclusion: In urine sediment analyzer, carryover% calculated by formula was found analytically sufficient, but it causes highly false-positive results due to diagnostic limit of hematuria (RBC > 3/hpf) is low. To prevent carryover in both urine analyzers; washing procedures should be revised and the diagnostic effect of carryover should also be taken into account by biochemists.

Keywords: Carryover; Carryover effect; False-positive hematuria; Automatic urine analyzer; Diagnostic.

Özet

Amaç: Laboratuvarımızda kullanılan idrar otoanalizöründe örneklerin kimiyasal ve sediment analizinde, analitik olarak kabul edilebilir sınırda olması rağmen yanlış pozitif hematüri tanısına yol açabilecek eritrosit bulaşmasını olup olmadığını tespit etmeyi amaçladık.

Metod: Hasta örneklerinden 24 adet makroskopik hematüri ve 48 adet eritrosit içermeyen idrar numunesi seçildi. Eritrosit içermeyen örnekler, makroskopik hematüri önünden tekrar analiz edildi. Bulaşma yüzdesi Broughton’un formülü \[\text{carryover\%} = 100 \times \frac{(b_1 - b_2)}{(a_2 - b_2)}\] ile hesaplandı. Bulaşma etkisinin sonuçları etkileyip etkilemediği Wilcoxon testi ile değerlendirildi.

Bulgular: İdrar kimiyasal analizöründe bulunan %67 oranında iken sediment analizinde ise %0,4 olarak bulundu. İdradık ihlas pozitif hematuri oranının hematüriden sonra çalışılan 1. örneklerde %87,5, 2. örneklerde ise %6,6 olduğu belirlendi. Her iki analizörde de hematüriden sonra gelen 1. örneklerin sonuçları, 2. örneklerin sonuçlarından istatistiksel olarak anlamalı yüksek bulundu (p < 0.001).

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Introduction

The main function of robotic liquid-handling systems is the pipetting of liquid specimens containing one or more analytes and reagents [1]. In these systems, pipetting can be executed in two ways in terms of usage of either fixed reusable tips or disposable tips [1]. Disposable tips are relatively expensive because they need to have required specifications [1]. As most of the assays require more than one pipetting step, it can be said that assay costs might increase more and more if disposable tips used [2]. Moreover, such tips become contaminated waste after their use, and require their own specialized robotic systems [3]. Due to the above mentioned reasons, the use of fixed reusable tips stands as an attractive alternative to the use of disposable tips [1]. The major problem of utilization of fixed tips in pipetting steps is the carryover [1]. Interassay carryover arises when residual quantities of assay materials that have remained after probe wash are carried into the protocol of the second assay through the probes [4]. These assay materials might be the sample itself, or any of analytes or the reagents that used throughout the assay as well [1].

Carryover causes systematic biases in assay results [4]. Sample-to-sample carryover (also referred as sample cross-contamination) can occur when a sample containing high analyte concentration followed by low (or zero) analyte concentration [1]. Carryover from the first to the second sample may cause the second sample to become false-positive [1].

Instrument manufacturers are required to perform various system performance validations either out of professional interest or regulatory requirement but routine laboratories do not verify the lack of sample carryover unless trying to explain discrepant results [3]. In development and clinical evaluation of any automatic analyzer, all potential sources of interassay carryover should be investigated and minimized [5, 6]. The effect of carryover on the precision and the accuracy of methods must be analyzed statistically [4]. When a specific interassay carryover identified, the clinical chemist has two prospective courses of action: to label the instrument with the estimated amount of detected carryover in order to identify the possible inaccuracy of the assay and to adjust the measured result as accurately as possible; or to modify the assay with the purpose of eliminating or substantially reducing the identified carryover (e.g., by increasing the volume of the corresponding washing step) [5].

The primary aim of this study was to find out whether there is significant carryover effect which causes false-positive hematuria in automatic urine chemistry and sediment analyzers. Our secondary aim was bring diagnostic pitfall of carryover – the effect of the analytical carryover on diagnosis – to biochemists’ notice.

Materials and methods

Assay procedure

In this study, two same samples containing high red blood cell (RBC) concentration placed to the rack prior to another two same samples containing zero RBC concentration also located to the same rack. The four samples analyzed, respectively (a1–a2–b1–b2) at H-800 urine chemistry analyzer (DIRUI, Changchun, P.R. China) [7].

The FUS-200 urine sediment analyzer (DIRUI, Changchun, P.R. China) has one pipetting step and two flow-cells which differs from the other analyzers. The first sample is imaged and counted at the first flow-cell, then the second sample is also imaged and counted at the second flow-cell. Due to the operation principle of FUS-200 urine sediment analyzer, we modified Broughton’s method to avoid potential positive bias originated from pipetting step. Four same samples containing zero RBC concentration placed to the rack sequential to the two same samples containing high RBC concentration located to the same rack as well. The six samples analyzed with respect to the placement row at FUS-200 urine sediment analyzer (a1–a2–b1–b2–b3–b4). Thus, the carryover between first, third and fifth (a1–b1–b3) samples which imaged at the first flow-cell evaluated amongst whereas the carryover between second, fourth and sixth (a2–b2–b4) samples which imaged at the second flow-cell assessed amongst as well. In order to prevent any other systematic biases (except for carryover) during the assay, the sample containing high RBC concentration was portioned in two tubes as the first two samples (a1–a2) and the other sample containing zero RBC concentration was portioned in four tubes as third, fourth, fifth and the sixth samples (b1–b2–b3–b4), respectively. Urine levels were equal in all these tubes (samples).

The CVs for the measurement of blood (H-800 urine chemistry analyzer) were 1.2% for negative control levels and 9.3% for positive control levels. The CV for the measurement of RBC (FUS-200 urine sediment analyzer) was 6.8%.
Selection of urine samples

Urine samples which used in this study are collected from adults’ urine specimens which came to Okmeydani Training and Research Hospital Clinical Biochemistry Laboratory for urinalysis with respect to obtained permissions from Okmeydani Training and Research Hospital Ethics Committee with the protocol number of 260 at 20/01/2015 and Turkish Drug & Medical Device Institution as well. The 24 samples with gross hematuria selected as containing high RBC concentration whereas another 48 samples that both chemical analyzer result of strip blood was negative and sediment analyzer result of RBC was 0/hpf (high power field) -that found out by manual control of microscopic images- selected as containing zero RBC concentration. Each test completed within 2 h following the collection of samples [8].

Statistical analysis

The design of this study was based upon 24 consecutive test analysis having 80% power with 5% type 1 error level to detect a minimum statistically significant difference of 20%. Power analysis was conducted by using G*Power 3.1 software. All statistical analyses were performed using the SPSS software package, Version 17 (SPSS Inc., Chicago, USA) for Windows.

Strip blood results of urine chemistry analyzer valued as ordinal variables in SPSS (negative = 0, trace = 1, +1 positive = 2, +2 positive = 3, +3 positive = 4). Then, the difference between two same samples (b1–b2) determined by Wilcoxon test.

The percentage of carryover was calculated and average of 24 results was taken into consideration in urine chemistry analyzer based on the analysis of the results of the samples with gross hematuria and the samples containing zero RBC, respectively.

Microscopic results of urine sediment analyzer had non-normal distribution. Therefore, the difference between two same samples (b1–b2) determined by Wilcoxon test.

As urine sediment analyzer has two flow-cells, 24 samples imaged and analyzed at each flow-cell. The percentage of carryover was calculated and average of 48 results was taken into consideration due to the analysis of each samples’ results at the same flow-cell.

The percentage of carryover (k) was calculated via the formula proposed by Broughton [7].

\[ k = \frac{b_1 - b_2}{a_1 - a_2} \times 100 \]

Table 1: Results of the blood negative samples which analyzed after the samples with gross hematuria at H-800 urine chemistry analyzer.

| Strip blood results | n  | Median | 25.p  | 75.p  |
|---------------------|----|--------|-------|-------|
| First samples analyzed after gross hematuria | 24 | +3     | +2    | +4    |
| Second samples analyzed after gross hematuria | 24 | –      | –     | +1    |

Figure 1: Mean of the strip blood results in H-800 urine chemistry analyzer.

Results

The first samples (b1) analyzed after gross hematuria had significantly higher results (p < 0.001) than the second samples (b2) analyzed after gross hematuria due to the observation of the results of the same negative samples in urine chemistry analyzer. Median of the strip blood results of the first samples analyzed after gross hematuria was +3 positive (Table 1).

Considering each result of the negative samples that analyzed subsequent to the samples with gross hematuria, the percentage of the carryover was calculated in urine chemistry analyzer. The average carryover of 24 results was explored as quite high, which was 67%. The means of the strip blood results can be seen at Figure 1.

False-positive (≥ +1) hematuria rate due to carryover was found as 91.6% (22/24) for the first samples analyzed after gross hematuria. On the other hand, the same rate was found as 20.8% (5/24) for the second samples analyzed after gross hematuria.

Among the results of the same samples containing zero RBC concentration that both analyzed after gross...
hematuria in urine sediment analyzer, the first samples (b₁ and b₂) had significantly higher (p < 0.001) results than the second samples (b₃ and b₄). Median of the microscopic RBC results of the first samples analyzed after gross hematuria was 12.5/hpf (Table 2).

The percentage of carryover was calculated in urine chemistry analyzer based upon the each result of zero RBC concentration samples which analyzed subsequent to the samples with gross hematuria at the same flow-cell. The average carryover of 24 results was explored as 0.44% at the first flow-cell whereas it was 0.35% at the second flow-cell (Figure 2).

False-positive (≥ 3/hpf) hematuria rate due to carryover was found as 87.5% (42/48) for the first samples (b₁ + b₂) analyzed after gross hematuria. The same rate was explored as 6.6% (3/48) for the second samples (b₃ + b₄) analyzed after gross hematuria (Table 3).

**Discussion**

There are many factors that can affect the accuracy of test results in the clinical laboratory, some more significant than others. The total analytical error associated with a result is usually defined as consisting of random error (imprecision) and systematic error (bias). Carryover is an example of an analytical systematic error that may tend to be overlooked. Sample carryover is classified under protocol specific bias at CLSI – EP10 guideline [9].

Carryover represents one of the performance tests in urine analyzers. However, for accurate interpretation of results in proper clinical evaluation, carryover should be considered in another way especially in case the analytes both have low reference values and may have high levels in patients. Obviously, samples that represent the highest concentrations likely to be found in an actual patient specimen should be tested so that the performance of the pipetting system is truly challenged [3].

According to the American Urological Association, asymptomatic microscopic hematuria is defined as three or more red blood cells per high-power microscopic field in urinary sediment from two out of three properly collected urinalysis specimens [10, 11]. In pediatric population, causes of asymptomatic hematuria must be investigated and then if necessary patients should be treated in accordance with diagnosis [12]. Interassay RBC carryover should be taken into account as it can be one of the causes of false-positive hematuria.

**Table 2:** Results of the zero RBC concentration samples which analyzed after gross hematuria at FUS-200 urine sediment analyzer.

| Microscopic RBC results | n | Median | Percentiles |
|-------------------------|---|--------|-------------|
|                         |   | 25.p   | 75.p        |
| First samples analyzed after gross hematuria | 48 | 12.5/hpf | 6.0/hpf |
| Second samples analyzed after gross hematuria | 48 | 0.5/hpf | 0.0/hpf |

**Table 3:** False-positive hematuria rates of the negative samples analyzed after gross hematuria at automatic urine analyzer.

| Results | Chemical | Microscopic |
|---------|----------|-------------|
|         | First samples analyzed after gross hematuria | Second samples analyzed after gross hematuria | First samples analyzed after gross hematuria | Second samples analyzed after gross hematuria |
| False-positive (n) | 22 | 5 | 42 | 3 |
| True-negative (n) | 2 | 19 | 6 | 45 |
| False-positive rate | 91.6% | 20.8% | 87.5% | 6.6% |

*DIRUI H-800 urine chemistry analyzer. *DIRUI FUS-200 urine sediment analyzer.
A literature search found relatively few papers discussing sample carryover especially of automatic urine analyzers. Only two studies which analyses carryover of the Sysmex UF-50 and UF-1000i urine sediment analyzers were found. Carryover rates for RBC counts of these analyzers were 0.352% and 0.07%, respectively [13, 14]. Okada H, et al. [13] and Wang J, et al. [14] interpreted these rates as sufficient but they didn’t evaluate the effect of that little amount of carryover on the urinalysis results just as false-positive hematuria rates.

In our study, RBC carryover percentage was found as 0.4% in urine sediment analyzer. Considering most of the automatic analyzers, carryover is <1%~2%, and usually this does not cause significant errors in routine analytical results [7]. However, as shown in our results, according to the analyze row of the same samples, the carryover of even 0.4% caused significant difference within the results. Despite carryover of 0.4% seems very good as an analytical performance test, the results are unacceptable diagnostically. The mean of RBC was found as 18/hpf whilst it was supposed to be lower than 3/hpf (can be seen at Figure 2) in urine samples’ microscopic results. This outcome may mislead the clinicians on diagnosis. The results indicated that carryover of 0.4% caused false-positive hematuria rate of 87.5% for the first samples analyzed after gross hematuria and 6.6% for the second samples analyzed after gross hematuria. In order to avoid false-positive results, urine specimens with gross hematuria should be analyzed alone; and then the analyzer is recommended to be washed with cleaning solution. In the long term pipetting and/or flow-cell washing quantity and procedures should be revised by the instrument manufacturers via similar studies.

In the literature, due to the lack of a study investigating the carryover of urine chemistry analyzers, we did not have the opportunity to compare our results. In addition, this finding showed that the adequate importance were not given by biochemists to this issue in spite of urinalysis is one of the most common screening tests performed in the clinical laboratories [15].

In this study, the percentage of blood carryover was found 67% in urine chemistry analyzer. This ratio is significantly higher than the acceptable carryover which is less than 1% of the analyte concentration for content uniformity in assays [16]. High carryover percentage decreases precision and accuracy of urine chemistry analyzer by leading high false-positive rates. Due to high carryover percentage, false-positive (≥1) hematuria rate was found 91.6% (22/24) for the first samples analyzed after gross hematuria and 20.8% (5/24) for the second samples analyzed after gross hematuria. False-positive results stand as an important issue because these cause unnecessary treatments, increased costs and time loss as well [17]. This case shows the importance of demanding the carryover percentage of semi-quantitative methods in addition to quantitative methods from manufacturers. High carryover percentage and the significant difference (p < 0.001) within the results according to analyze row of the same samples indicate the volume of pipette washing step is insufficient. In order to solve these problems, either volume of the pipette washing step or the number of washing steps should be increased. To avoid false-positive urinalysis results, urine specimens with gross hematuria should be analyzed alone and then washing the analyzer with cleaning solution is recommended.

Due to these results each laboratory, on their own terms, should determine analyze procedure and rejection criteria of the samples containing high analyte concentration. Otherwise, the usage of disposable tips in pipetting steps should be considered in the analyzers which have high carryover effect.

Boneno J, et al. [18] discussed that in some cases, a change in result values because of carryover% may be analytically insignificant, but clinically significant, depending on the specific analyte, the normal reference range, the medical decision level, and other considerations. Conversely, some change in result values may seem to be analytically significant but ultimately may be clinically insignificant. That was the case in this study. Thus, we have given a concrete example to this important issue with urinalysis which is the one of the most common screening tests. In the light of this debate, we entitled this argument as diagnostic pitfall of carryover for terminology.

It is understood that evaluation of analytical carryover is not adequate for some parameters in terms of analyzer performance. Therefore, according to the rules of evidence-based medicine, diagnostic effect of carryover should also be used in clinical laboratories. The amount of the analyte carryover should be used instead of carryover percentage in the parameters that especially their reportable ranges are much higher than the reference values. Due to little amounts of change in these parameters is so crucial for diagnosis and follow-up. Hence, evaluation of the diagnostic effect of carryover should be carried out as it is so essential to prevent false-positive test results.

Conflict of interest statement: There are no conflicts of interest among the authors.
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