Evaluating the consistency of urinalysis results from the Urisys 1800 and microscopy

Jun (Rebecca) Lu, BMLSc, MNZIMLS, Medical Laboratory Scientist1 Xiaoyi (Michael) Ding, BCom, Tutor in Applied Statistics2

1Automation Department, Southern Community Laboratories, Southland Hospital, Invercargill
2Victoria University, Wellington

Abstract

Objectives: Discrepancies of measurements for urine analytes using various methods have previously been described. Due to its importance as an indication of pathological conditions, the reliability of results are questioned. The aim of this study was to determine whether differences observed were statistically significant and to discuss the common factors involved that could potentially explain the discrepancies in order to assist clinicians to make a diagnosis.

Method: Urine samples (n=197) were tested by three methods: Urisys 1800 (Roche Diagnostics), manual reading of the test strip and by microscopy. Three of the most clinically significant analytes (red cell count, white cell count and protein quantity) were compared by applying the 95% confidence interval.

Results: We were 95% confident that the Urisys 1800 gave higher values than manual strip readings, relating to white cell, red cell and protein quantity. No significant difference was detected for white cell counts based on paired data obtained from Urisys 1800 and microscopic urinalysis, while red cell counts by microscopy were significantly greater than by the Urisys 1800.

Conclusion: Based on the principles of different testing procedures, cell lysis and interferences from other urinary components and substances may contribute to the discrepancies observed. In combination with individual patient’s clinical details, it is possible for laboratory staff to advise clinicians with a better interpretation of the urinalysis results.

Key words: Urisys 1800, microscopic analysis, white cell count, red cell count, protein quantity.


Introduction

The Urisys 1800 (Roche Diagnostics) is a sensitive and specific analyzer for urinalysis, measuring various urine analytes semi-quantitatively, including specific gravity, pH, leukocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin and blood (1). It is commonly referred to as “urine dipstick” and the procedures are simple and quick.

It is known that changes in body physiological conditions during disease states may lead to relevant alterations of the urinary components. By monitoring those by urinalysis an indication of disease conditions is possible and further clinical tests can be followed up. For instance, an elevation of urine glucose and/or ketones could be a sign of diabetes mellitus; while an increase in the leukocyte count and nitrate may suggest a urinary tract infection (UTI) or a kidney disorder (2).

Test strips are the media containing pads embedded with specific reagents and the amount of an analyte in the sample is proportional to the colour intensity developed on the pad via chemical reactions (2). The concentration value is then converted from the electro-optical measurement obtained by a detector. Each measurement is adjusted in comparison to a reference strip and the intrinsic colouration of the urine is taken into account to increase accuracy of the results (1). However, test strips can also be read manually.

In clinical laboratories, microscopic analysis is conducted either on urine samples with at least one significantly abnormal analyte value from dipstick or basically on all urine specimens. White cell count and red cell counts are directly estimated in the counting chamber. However, it has been previously shown that results obtained by microscopy do not always agree with those from the Urisys 1800. The aim of this study was to compare the accuracy of the Urisys 1800 to those of manual strip reading and microscopic examination.

Materials and methods

The data were collected in September 2007 in Southland Hospital laboratory, which consisted of urine samples from both inpatients and outpatients. The main type of specimens were mid-stream urine and clean catch urine samples. Each specimen had relevant information recorded, including gender and age of the patient, time elapsed till its analysis. Significant clinical details were also noted, mainly pregnancy and UTI. The analyzer used was a Urisys 1800 with Combur10® Test M test strips (lot number: 11379208) and microscopic analysis was performed on an Olympus BX40CY microscope. Two liquidhek™ urinalysis controls (lot number: 61281 and 61282) were run daily on the Urisys 1800 before batches of patient samples.

Preferably, urinalysis was performed within 2 hours of collection, in cases where an immediate urinalysis was not possible, samples were refrigerated at 2-8°C for up to 24 hours, according to the guidelines of sample collection and transportation (3). Refrigerated urine samples were returned to room temperature before analysis and direct exposure to sunlight and additional preservatives was avoided. After a thorough mix of the urine, a test strip was dipped completely into the sample and placed onto the test strip tray of Urisys (1). The same procedure was repeated but the strip was read manually by referring to the scales on the strip container based on the colour intensity developed. White cell count was read between 60s and 120s while all other analytes were read immediately after 1 minute. Importantly, any colour development along the edges of a pad only was regarded as negative, which occurred frequently for protein in this study. For both automatic and manual testings, only white cell, red cell and protein results were recorded.

Each sample was then loaded onto a counting chamber for a
microscopic examination of white cell and red cell counts. The whole area was counted under x40 magnification when the cell number was low, whilst only a representative central square was counted and multiplied by a common factor of 10 if the count was relatively high (4). All three methods were performed by the 1st author to reduce potential inter-personal bias. Results are presented as the number of cells per µl for white cells and red cells, and g/l for protein (1).

As we noticed that the assigned concentration intervals of both white cell and red cell for microscopic measurements differed from the ones from Urisys 1800 and the manual strip reading, paired comparison method was applied for analysis (6). Five sets of paired data of either automatic vs manual or automatic vs microscopy for a particular analyte were set up, and the mid-point value of each concentration interval was selected for calculation (5). For each set, a two-tailed 95% confidence interval (CI) of the difference was calculated (5).

Results

The total number of urine samples processed in this study was 221. Among those, 19 did not have collection time stated, four were analyzed after 24 hours and one showed severe haematuria thus rendering microscopic analysis impossible. Other mild or moderate blood-stained urines were not excluded as they are constantly encountered at clinical settings. Therefore, the final sample size was 197 and 68% of them were from female patients. The age of the patients ranged from 1 month to 99 years old with an average age of 53.7 years.

Eight sets of data collected from control level 1 were very precise for all three analytes of interest; however, one set of white cell count and protein quantity from control 2 were quite different from the rest. Tables 1 and 2 below demonstrate the 95% CIs for all five sets of data and the large standard deviation is indicative of the wide spread of data. Readings from the Urisys 1800 are significantly higher than manual strip readings. As shown in Table 2, there is no evidence to support that white cell counts from the Urisys 1800 are different from microscopic analysis. However, there is evidence that the red cell count by microscopy is indicative of elevated results.

Table 1. Urisys 1800 vs manual readings

<table>
<thead>
<tr>
<th></th>
<th>Mean of difference</th>
<th>Standard deviation of difference</th>
<th>Standard error of difference</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>8.89</td>
<td>53.34</td>
<td>3.80</td>
<td>1.44 to 16.34</td>
</tr>
<tr>
<td>RBC</td>
<td>14.63</td>
<td>22.25</td>
<td>1.585</td>
<td>11.52 to 37.21</td>
</tr>
<tr>
<td>Protein</td>
<td>0.051</td>
<td>0.16</td>
<td>0.0114</td>
<td>0.029 to 0.107</td>
</tr>
</tbody>
</table>

Table 2. Urisys 1800 vs microscopy

<table>
<thead>
<tr>
<th></th>
<th>Mean of difference</th>
<th>Standard deviation of difference</th>
<th>Standard error of difference</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC</td>
<td>-9.58</td>
<td>78.93</td>
<td>5.62</td>
<td>-20.6 to 1.44</td>
</tr>
<tr>
<td>RBC</td>
<td>-14.20</td>
<td>63.62</td>
<td>4.53</td>
<td>-23.08 to -5.32</td>
</tr>
</tbody>
</table>

Since a significant difference was found when performing statistical analysis on red cell count data from the Urisys 1800 and microscopic urinalysis, Table 3 assists in the interpretation of the discrepancy. Table 3 assists in the interpretation of the discrepancy. Urine samples with an Urisys 1800 reading of 150-250 cells/µl have a whole spectrum of interval readings from microscopy analysis. Also, there are 19 specimens which tested negative by the Urisys 1800 but harvested a cell count range of 0-10 cells/µl. However, approximately 60% of all the samples have given similar results by both methods.

Table 3. Comparison of RBC data by Urisys 1800 and microscopy

<table>
<thead>
<tr>
<th></th>
<th>Microscopy readings</th>
<th>Urisys readings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Negative</td>
<td>0-10</td>
</tr>
<tr>
<td>WBC</td>
<td>Negative</td>
<td>0-10</td>
</tr>
<tr>
<td>RBC</td>
<td>0-10</td>
<td>14</td>
</tr>
<tr>
<td>Protein</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Discussion

The control level 2 contains higher concentrations of all analytes than the control level 1, and the control level 2 is more sensitive to storage condition and testing procedures which could result in false measurements if performed inadequately. For example: insufficient mixing of control material before analysis, gradual deterioration of components during long storage duration, control material not refrigerated promptly after analysis, etc. The day-to-day variation of control 2 readings had been noticed by laboratory stuff before this study and a change of the storage condition for control material has subsequently been applied. Smaller aliquots of each control that is sufficient for a single analysis were prepared, capped and stored at 2-8°C. Each aliquot is used once only and discarded afterwards. The random variation was then noticed less frequently.

The higher concentration readings for white cell, red cell and protein by the Urisys 1800, compared with manual readings, may be related to the principle of measurement. A testing pad with a colour intensity developed that is slightly darker than a reference scale is classified into the same concentration range as the reference scale by manual readings (1,2). However, the Urisys 1800 analyzer categorises it into the next darker scale, resulting in a higher concentration interval (2). Thus, the Urisys 1800 tends to give greater values overall, regardless of the types of analytes. Additionally, manual reading of a test strip is relatively subjective and inter-personal variation can be large. Moreover, another common problem encountered, especially by manual strip reading, is the colouration of urine samples. Blood-stained specimens can be caused by bladder and/or kidney conditions, while abnormally coloured urine may be a result of liver disease, or intake of certain medications or foods (9). All those render it difficult to provide an accurate manual reading as the reaction pads are stained by the intrinsic colour of the urine and the true intensity is obscured (1). In contrast, the effect of urine colouration is minimised via comparison to a reference strip during automatic analysis by the Urisys 1800 (1).

White cell counts measured by the Urisys 1800 are directly proportional to the amount of esterase present in the urine, which means both intact and lysed white cells are counted (2). Several factors are found to potentially influence its measurement. False negative white cell concentration may occur due to the presence of leukocyte esterase inhibitors (2,10). Glycerol and/or ketonuria can result in falsely low measurements which could be an issue when analysing specimens collected from diabetic patients and other supplementary tests may be required to exclude suspected medical conditions (9). High urine protein and usage of certain oxidizing drugs may also lead to a false negative white cell count (9). As we have found in study, four urine samples with significant proteinuria had much lower white cell counts by dipsticks than by microscopy. On the other hand, false positive white cell counts are
usually caused by contamination, usually indicated by the presence of high numbers of epithelial cells and a repeat specimen may thus be necessary (9). In microscopic examinations, only intact white cells are counted and we would expect to have a lower white cell count from microscopy than the dipstick method. However, no significant statistical differences were found and a combination of several interference factors, as mentioned above, might have been involved in our study. As adopted from the Urisys 1800 performance evaluation report, false negatives were seen infrequently from the Urisys 1800 (7).

Similar to the white cell count, besides intact cells, lysed red cells are also taken into account by the Urisys 1800 dipstick analysis (2, 7). A colour-producing oxidation is catalysed by haemoglobin released from red cells and also by myoglobin that may be contained in the specimen (2). Thus, red cell counts may be falsely high if patients have myoglobinuria (9). Falsely positive or elevated measurements from the Urisys 1800 may also be due to the presence of oxidizing agents, haptoglobulin and bacterial peroxidise in the urine, as well as the acidic property of the urine sample (pH<5.1) (2,7,9). The inhibitory effect from ascorbic acid (vitamin C) is minimized by the incorporation of iodate in the test region on the test strips (2). Proteinuria was found by other studies to confer a false negative red cell count though this was not observed in our study (9). Interestingly, the red cell count by microscopy conferred statistically higher readings, although only intact red cells are counted. Several possible causes might be relevant; when fatty granules of variable sizes and/or yeast cells that resemble small and oval red cells are seen under microscope, the differentiation may not be easy, resulting in inaccurate red cell counts. Over-estimation may partially explain us observing 19 urine samples showing 0-10 cells/µl by microscopy that were negative by the Urisys 1800 (Table 3). Furthermore, gradual occurrence of red cell lysis was observed on samples with a high concentration of 150-250 cells/µl by the Urisys 1800, which is evidenced by a whole range of concentrations found by microscopy.

The Urisys 1800 analyser has been widely applied in clinical settings. When a discrepancy of the analytes between different testing methods is noticed, further information from the clinicians is essential. The possible presence of interfering substances due to either medications or existing conditions of the patient can be relevant to differentiate the reliability of test results, in order to assist clinicians in reaching a diagnosis or monitoring treatment. A limitation of our study is that the sample size might need to be larger and include samples from random community patients to ensure a better interpretation of the entire population with minimal bias. Urinary white cells and red cells only remain stable to ensure a better interpretation of the entire population with minimal bias. Urinary white cells and red cells only remain stable for up to 4 hours at refrigerated condition, which indicates a possible occurrence of continuous concentration alteration during urinalysis for a proportion of samples in our study (2). Moreover, a low specific gravity and/or an alkaline pH can speed up cellular lysis, which, unfortunately, was not investigated in this study (2, 10).

In conclusion, automatic urinalysis and microscopic examination of clinical urine specimens are the major techniques used in medical laboratories for measuring urine analytes that may be disease associated. The potential inconsistencies of the results obtained were tested in our study. Although no significant differences were found for white cell counts between the methods, microscopy gave higher red cell counts than the Urisys 1800. Gradual cell lysis and the involvement of interference factors are considered as the main contributors to the difference observed. Further studies on similar fields are fundamental to ensure the reliable determinations of urinary analytes.

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Address for correspondence: Rebecca Lu, Automation Department, Southern Community Laboratories, Southland Hospital, P O Box 828, Invercargill. Email: junlu570@hotmail.com

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