Comparative Analysis of Sensitivity to Blood in the Urine for Urine-based Point-of-Care Assays (UBC rapid, NMP22 BladderChek and BTA-stat) in Primary Diagnosis of Bladder Carcinoma. Interference of Blood on the Results of Urine-based POC Tests

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Abstract. *Background: According to guidelines, the primary diagnosis of bladder carcinoma is symptom oriented. This means that diagnostic testing is indicated for macrohaematuria, chronically recurrent microhaematuria and chronic bladder urgency. This study tests the suitability of three point of care (POC) test systems, UBC rapid, NMP22 BladderChek and BTA stat, available on the market, with respect to interference due to blood contamination in urine samples. Materials and Methods: Urine samples were obtained from voluntary asymptomatic individuals without a history of bladder cancer. A specimen negative in all test systems was selected for further study. This sample was treated with fresh heparinized blood in a 1:10 ratio and then titrated in a dilution series. All the urine samples and their consecutive test results were photographed and a urinalysis was performed on each sample. Results: In none of the samples of the dilution series did UBC rapid or NMP22 BladderChek show a false-positive result due to blood contamination. In contrast, with the BTA stat testing system, false-positive results were obtained from all samples with macrohaematuria and with densities up to 150 erythrocytes/μl, indicating a suspected tumour, whereas the sample was actually proven to be tumour free. Conclusion: For the primary diagnosis of bladder carcinoma, neither the UBC rapid nor the NMP22 BladderChek POC test systems are sensitive to the presence of blood in the urine, whereas BTA stat consistently yields false-positive results due to cross-reactivity to macrohaematuria and microhaematuria up to a density of 150 erythrocytes/μl, thus this system should not be employed for this examination.*

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Key Words: POC test, urine, blood contamination, complement factor, tumor marker, haematuria.

The rate of new cases of bladder carcinoma in Germany is constant at nearly 30,000 individuals every year (1). The vast majority of these patients are diagnosed as a consequence of their symptoms. To date, no preventative diagnostic testing has been implemented for bladder carcinoma and such testing is not currently recommended in any guidelines from national or international specialist committees (2). The classical symptom triad for bladder carcinoma consists of acute macrohaematuria (3), chronically recurrent microhaematuria (4) and persistent symptoms of urinary urgency (5). The three sets of symptoms occur at a frequency from 60% to 30% to 10%, respectively. From this fact, it is apparent that any diagnostic procedure employed for the primary diagnosis of bladder carcinoma must be insensitive to the presence of blood in order to rule out interference with the test system through blood. For this reason, we tested all three of the (POC) tests available on the German market with normal urine samples in a series of dilutions with artificially added blood.

Materials and Methods

Initially, multiple urine samples were obtained from asymptomatic volunteers who were younger than 55 years of age and had no history of bladder cancer, and tested using all three systems. Any individual who showed normal findings for all parameters was asked to provide a urine sample for each of four subsequent experiments. In each instance, a sample of 200 ml was obtained and in an initial pilot experiment with this urine, a 1:10 dilution series with heparinized blood was prepared over nine dilution steps. After this

Figure 1. *Initial dilution series 1:10 over 9 dilutions plus pure urine sample.*

pilot, the experiment was repeated three times, where the initial sample and the second diluted sample were diluted 1:10 with blood, and all subsequent dilutions were prepared at 1:2 dilutions over eleven following dilution steps, resulting in 13 dilutions plus pure undiluted urine. Urinalysis was performed on all samples using a Combur-10 test strip. The parameters included for measurement were urine specific gravity, pH, leukocytes, nitrite, protein, glucose, ketones, urobilinogen, bilirubin and erythrocytes. Overt infection was ruled out in all experimental samples using an Uricult test.

The pilot urine-blood dilution series were photographically documented. All three test systems, UBC rapid, NMP22 BladderChek and BTA stat were employed according to the manufacturer's instructions. For each test, a fresh urine sample taken from the dilution series was applied at the suggested volume in the test cassette and incubated for the prescribed time period. Subsequently, each test result was read and documented.

Results

All of the original urine samples were free of infection, blood contamination, and reproducibly gave no indications of tumor in all test systems. A simulation of haematuria was initially produced by the addition of 500 μl of heparinized blood to 4.5 ml of urine, and then diluted by 1: 10 through 13 dilution steps. The pathological changes in the urine produced by the addition of blood were documented in the urinalysis (Table I). In addition, the pilot dilution series was photographically documented (Figure 1).

Test results for each test system were read and photographically documented for all stages of dilution. To ensure a better overview, in all figures presenting the dilution series for all investigated test systems we selected the dilutions 1, 3, 5, 8, 10 and the result of pure urine. This revealed that for all dilutions with visible blood mixing, and in the more intensively contaminated microhaematuria dilutions (up to 150 erythrocytes/μl), the results were always positive for suspected bladder cancer for BTA stat, whereas all other samples with higher levels of dilution and the pure urine sample were negative for bladder cancer (Figure 2).

For UBC rapid and NMP22 BladderChek, however, the test results were negative throughout all the test cassettes, and this was repeated across the entire range of dilutions, as well as for the pure urine sample. At maximal concentrations of blood in the urine, the only problem that arose was that the erythrocytes fell with the sedimentation flow and arrived at places in the test cassettes, where the marking lines were partially covered. This made interpretation impossible. However, no cross-reactivity of the antigen was observed with the blood-contaminated sample (Figures 3 and 4).

Discussion

Using this experimental approach, we conclusively demonstrated that both POC test systems, UBC rapid and NMP22 BladderChek, are not sensitive to blood contamination in urine. With massive clinical macrohaematuria, it is possible that as a coagulum forms, it is only possible to use the test cassettes to a limited extent, since the erythrocyte sedimentation reaches the reading area for the test system, and covers the reading line; however, no false-positive findings occur due to cross-reactive binding of blood components. For this reason, both test systems are well suited, in principle, for primary diagnosis in symptomatic patients. Blood in the urine does not represent an exclusion criterion for either of these test systems.

By contrast, the BTA stat test was found to be falsely positive in the presence of macrohaematuria, and this critical finding persists up to a density of 150 erythrocytes/μl in the microhaematuria range. This finding corresponds to results from previous studies regarding this parameter(6). It results from an interaction between blood

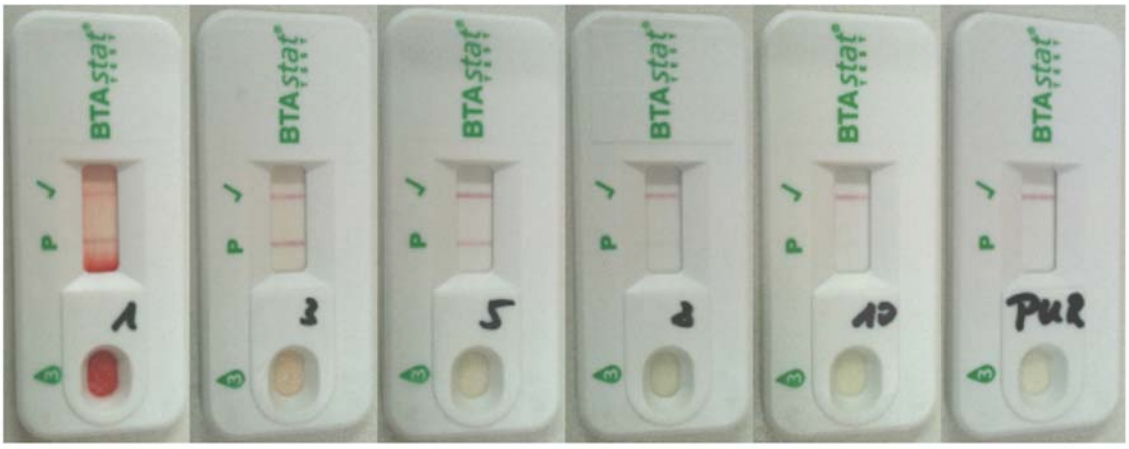

Figure 2. *Photo documentation of BTA stat by dilution.*

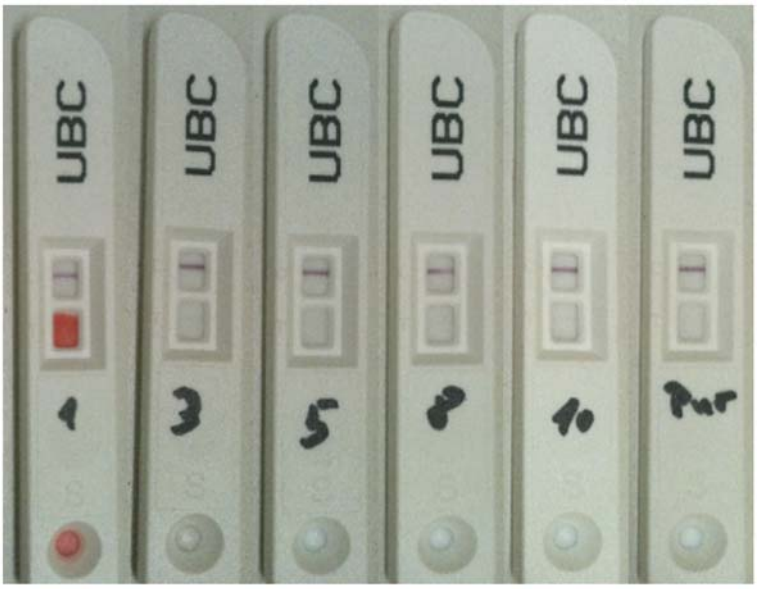

Figure 3. *Photo documentation of UBC rapid by dilution.*

Figure 4. *Photo documentation of NMP22 BladderChek by dilution.*

Table I. *Presentation of urine status and POC-Test results in correlation to the urine-blood dilution series.*

Code	Dilution	Weight density	pH	Leuco-cytes	Nitrite	Protein	Glucose	Ketone	Urobi-linogen	Bilirubin	Erythro-cytes	BTA stat	NMP22	UBC rapid
1	Macrohaematuria 1:10	1.025	5	25/µl	Pos	150/µl	Neg	Neg	Neg	Neg	250/µl	Pos	Not readable	Not readable
2	Macrohaematuria 1:100	1.025	5	Neg	Pos	150/µl	Neg	Neg	Neg	Neg	250/µl	Pos	Neg	Neg
3	Macrohaematuria 1:200	1.025	5	Neg	Neg	75/µl	Neg	Neg	Neg	Neg	250/µl	Pos	Neg	Neg
4	Macrohaematuria 1:400	1.025	5	Neg	Neg	75/µl	Neg	Neg	Neg	Neg	250/µl	Pos	Neg	Neg
5	Macrohaematuria 1:800	1.025	5	Neg	Neg	25/µl	Neg	Neg	Neg	Neg	250/µl	Pos	Neg	Neg
6	Macrohaematuria 1:1600	1.025	5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	250/µl	Pos	Neg	Neg
7	Microhaematuria 1:3200	1.025	5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	250/µl	Pos	Neg	Neg
8	Microhaematuria 1:6400	1.025	5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	150/µl	Weakly pos pos	Neg	Neg
9	Microhaematuria 1:12800	1.025	5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	50/µl	Neg	Neg	Neg
10	Microhaematuria 1:25600	1.025	5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	10/µl	Neg	Neg	Neg
11	No haematuria 1:51200	1.025	5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
12	No haematuria 1:102400	1.025	5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
13	No haematuria 1:204800	1.025	5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg
Pure	Pure urine	1.025	5	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg	Neg

components and the antigen that is employed in the BTA stat test. According to information from the manufacturer and the primary published authors, the BTA stat test measures a protein that is similar to a complement factor(7). Since the complement system is present in blood, what is likely to be taking place here is a cross-reaction between a complement factor from the blood and a tumour-associated complement factor-like protein. As a result, the specificity of this test is completely abolished in the presence of macrohaematuria, and in the range of higher erythrocyte densities with microhaematuria, it results in a false-positive finding.

From a clinical perspective, one must point out that the BTA stat assay is not appropriate for the primary diagnosis of bladder carcinoma, once macrohaematuria or even a higher level of microhaematuria is present. Since this is true for more than 75% of the cases where diagnostic testing is indicated, BTA stat is unsuitable for the diagnosis of bladder carcinoma because of its cross-reactivity. Should there be no macrohaematuria present in the clinical setting, it would still be necessary to obtain a complete urinalysis along with the screening test in order to be able to definitively classify the test results.

Acknowledgements

This study was made possible through support from the firm Concile GmbH, which provided all the test systems.

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Received September 15, 2011
Revised October 24, 2011
Accepted October 26, 2011