

# Bladder EpiCheck Is A Sensitive and Specific Novel Molecular Diagnostic Tool for Monitoring Of Bladder Cancer Patients

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## Introduction

Bladder cancer (BC) is the 5<sup>th</sup> leading cancer in Europe<sup>5</sup>, and one of the cancers with the highest lifetime cost due to high recurrence rate and the need for ongoing invasive monitoring <sup>6</sup> (i.e. cystoscopy). Cystoscopy is usually considered as the gold standard for bladder cancer detection but it is invasive and relatively expensive. Therefore, in the surveillance of papillary low-grade tumors, a noninvasive, highly sensitive, and specific bladder cancer marker could decrease the frequency of cystoscopies, thereby improving patient quality of life. In high-grade disease, increased sensitivity of markers might lead to earlier detection of tumor recurrence, resulting in improved patient survival<sup>7</sup>.

These, along with the discomfort and anxiety of frequent cystoscopies, have led to much effort in developing alternative, less-invasive methods for BC surveillance<sup>8</sup>, mostly urine bound tests. There are currently several urine tests in clinical use, but none of them has shown high enough performance (sensitivity, specificity and predictive values) to decrease the frequency of cystoscopy in the monitoring guidelines, or is widely used for this purpose. It is even argued that the poor performance, marginal clinical utility, and potential harm of the currently available urine tests make them inadequate for regular clinical use<sup>9</sup>.

Therefore, a urine test with a high sensitivity and specificity can provide a clinically beneficial tool in BC monitoring for both low grade and high grade tumors.

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<sup>5</sup> GLOBOCAN 2012: estimated cancer incidence, mortality, and prevalence worldwide in 2012. International Agency for Research on Cancer Web site. <http://globocan.iarc.fr>.

<sup>6</sup> K. D. Sievert et al, World J Urol (2009) 27:295–300

<sup>7</sup> Bernd J. Schmitz-Dräger et al, Molecular Markers for Bladder Cancer Screening, Early Diagnosis, and Surveillance: The WHO/ICUD Consensus, Urol Int 2015;94:1–24

<sup>8</sup> van der Aa MN, Steyerberg EW, Sen EF, et al. Patients' perceived burden of cystoscopic and urinary surveillance of bladder cancer: a randomized comparison. BJU Int 2008;101:1106–1110

<sup>9</sup> Joseph J. Fantony, and Brant A. Inman, It May Be Time to Abandon Urine Tests for Bladder Cancer, JNCCN, Volume 13 Number 9 1163-1166

## Bladder EpiCheck

We evaluated a new test for monitoring BC patients post TURBT. The test – Bladder EpiCheck (Nucleix Ltd., Rehovot, Israel) is performed on voided urine samples and identifies patient in whom cancer has recurred.

Bladder EpiCheck is a new molecular biology assay for detection of BC in voided urine samples, based on identification of DNA methylation changes associated with BC in a panel of 15 genomic biomarkers. The assay generates a numerical EpiScore (0-100) reflecting the overall methylation level in the urine sample. A case is regarded as positive when the EpiScore is  $\geq 60$ . The aim of this study was to assess the sensitivity and specificity of the assay in a population of patients with a history of BC.

## Materials and Methods

### Patients and Urine Samples

Voided urine samples from 222 patients with history of BC undergoing routine surveillance were collected to be processed with the Bladder EpiCheck kit. The urine samples were stored, without any preservative, in normal refrigeration for up to 5 days from the time collected per the EpiCheck protocol.

The patients donating the urine underwent routine follow up. Out of the 222 patients 40 (18%) had biopsy-proven recurrent bladder cancer, and the remaining 182 cases were negative. 175 of these samples underwent cytology evaluation. The positive cases distribution was as follows (note – some cases had more than one stage category):

Stage	# of cases
Ta	16
T1	11
T2	3
CIS	12

The grade distribution of the positives was:

Grade	# of cases
Low grade	21
High Grade	19

## Method

All urine samples were analyzed with the Bladder EpiCheck kit. The samples underwent the following steps:

1. **Creating a cell pellet from the urine sample.** The urine sample is centrifuged such that the cells (both normal and cancerous, if present) are separated from the urine liquid.
2. **DNA extraction.** DNA is extracted from the cell pellet using Bladder EpiCheck DNA extraction kit
3. **Digestion of extracted DNA.** Extracted DNA is digested using a methylation-sensitive restriction enzyme, which cleaves DNA at its recognition sequence if it is unmethylated (methylated DNA remains intact).
4. **Real-time PCR amplification.** Real-time PCR amplification is performed in a 96-well plate using the Applied Biosystems 7500 Fast Dx instrument (Thermo Fisher). The samples are prepared for the PCR assay using the Bladder EpiCheck test kit

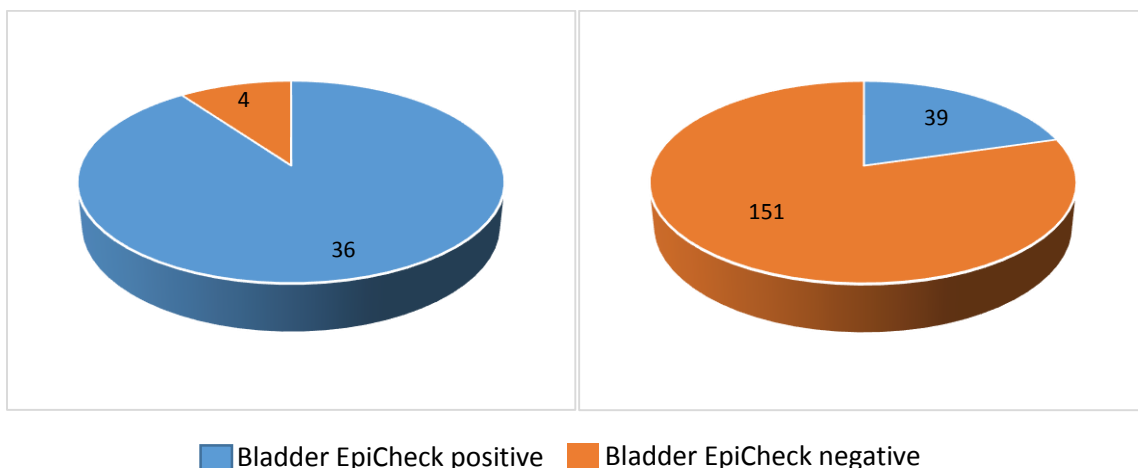
At the end of the PCR run the output file of the PCR was analyzed by the Bladder EpiCheck software. The software algorithm first ensures that the control samples of the kit are OK – meaning that the run went well and can be analyzed. If the control samples are OK, the software proceeds to analyze the patient samples. If any of the controls of a patient sample fails, this sample is not analyzed and a diagnosis is not given. For those samples that passed the internal control validation the software calculates their **EpiScore** – a number between 0 and 100 representing the overall methylation level of the sample at the panel biomarkers. If the EpiScore is equal or above 60 then the patient is regarded Positive (indicating a high risk for bladder cancer), otherwise the patient is regarded Negative (indicating a low risk for bladder cancer).

## Results

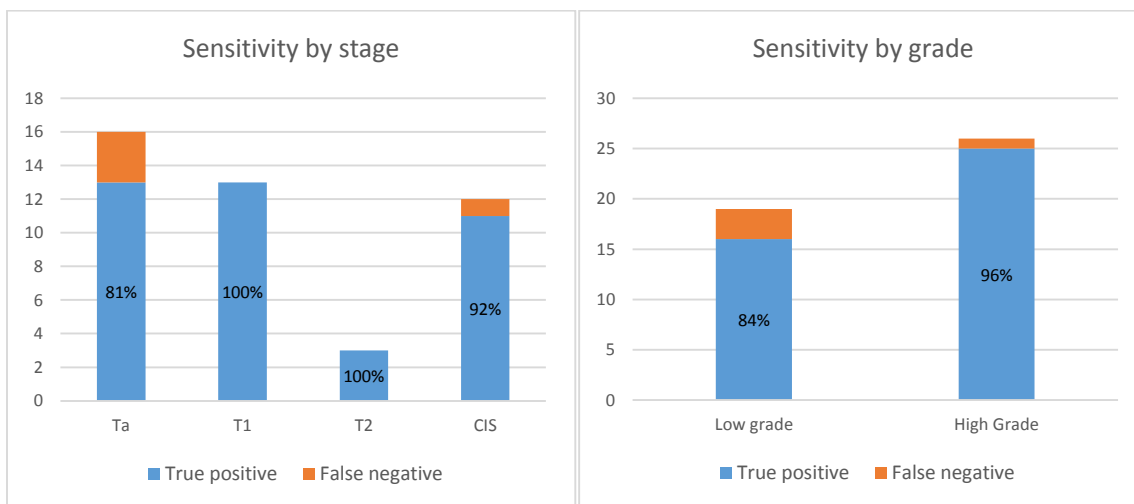
### Sensitivity and Specificity

Bladder EpiCheck showed overall sensitivity of 90%, correctly diagnosing 35 out of the 39 positive cases, and overall specificity of 83%, correctly diagnosing 151 out of the 182 cancer free patients.

Bladder EpiCheck Sensitivity and Specificity



The sensitivity and specificity breakdown per stage and grade are shown in the graphs below:



### Negative Predictive Value (NPV)

The negative predictive value in this study was 97.4% (based on the prevalence of recurrence in this cohort, which is 18%).

Analysis based on the same level of sensitivity and specificity of this study (i.e. 90% sensitivity and 83% specificity) assuming different levels of prevalence yields the following results:

Prevalence	10%	18%	20%	30%
NPV	98.7%	97.4%	97.1%	95.1%

### Comparison with cytology

Cytology evaluation results were available for 175 patients of the study cohort. Cytology correctly diagnosed 5 out of 13 bladder cancer patients (38% sensitivity), and correctly diagnosed 155 out of 162 cancer free patients (96% specificity). Bladder EpiCheck detected all tumors that were detected by cytology, and all of the tumors that were missed by Bladder EpiCheck were also missed by cytology.

### EpiScore distribution

#### By grade

We analyzed the correlation between tumor grade and the EpiScore. The average EpiScore of the high grade tumors was 85 and for the low grade tumors was 69. Statistical analysis using the Kolmogorov-Smirnov test showed that the EpiScore is significantly correlated with tumor grade ( $p=0.006$ ).

### By stage

We calculated the average EpiScore for each of the stages of the tumor:

Stage	Average EpiScore
Ta	68.6
T1	84.3
T2	90.7
CIS	81.8

Due to the fact that the sample size is relatively small it is hard to show statistical significance. Nevertheless, statistical analysis using the Kolmogorov-Smirnov test showed statistical significant difference between the Ta and T2 scores ( $p=0.0077$ ) and between Ta and CIS ( $p=0.0155$ )

### Test success rate

Over 98% of the urine samples that were tested in this study were successfully analyzed by Bladder EpiCheck.

### Discussion

In this study we demonstrated that Bladder EpiCheck has high sensitivity, specificity and negative predictive values, higher than other existing non-invasive tests for BC<sup>10</sup> and is therefore a highly effective tool for monitoring BC patients.

The assay numerical measure – the EpiScore – showed statistically significant correlation with tumor stage and grade. This also suggests that Bladder EpiCheck may enable a more fine-tuned analysis of bladder cancer tumors.

Additionally, we compared the results of the Bladder EpiCheck assay with the cytology results. The specificity and sensitivity of cytology in our study were similar to those quoted in the literature<sup>8</sup>. It is noted that all positive cases identified by cytology were also identified by Bladder EpiCheck

### Conclusion

The study results show that the Bladder EpiCheck is a sensitive and specific assay for the detection of bladder cancer in voided urine samples.

The high sensitivity, specificity and negative predictive value of the assay, particularly in the high risk bladder cancer population, promotes a substantial adjunctive role in the follow-up of such patients.

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<sup>10</sup> Bernd J. Schmitz-Dräger et al, Molecular Markers for Bladder Cancer Screening, Early Diagnosis, and Surveillance: The WHO/ICUD Consensus, Urol Int 2015;94:1–24