Clinical Validation of a Urine-based *PENK* DNA Methylation Assay (EarlyTect BCD Plus) for Primary and Recurrent Bladder Cancer Detection

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Introduction

For patients with hematuria, current guidelines follow a risk based approach to screening for bladder cancer by cystoscopy. However, in practice, there are two challenges:
1) a substantial number of invasive procedures are performed in patients without malignancy; 2) many patients at elevated risk avoid cystoscopy.

In addition, because of the high rate of BC recurrence, the recommendation is for surveillance by cystoscopy at frequent intervals, which can be challenging for patients. These issues could be mitigated with diagnostic tests based on urinary tumor biomarkers.

However, existing urine-based assays have shown limited clinical adoption due to suboptimal sensitivity for early-stage/low-grade disease or limited specificity.

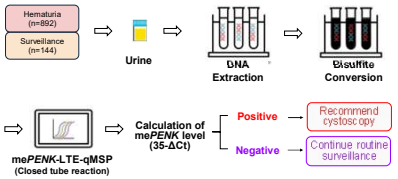
There remains an unmet need for non-invasive biomarker tests that can:

- Accurately stratify BC risk to rule-out or rule-in cystoscopy
- Maintain high sensitivity for clinically significant disease
- Support guideline-concordant triage and surveillance strategies

EarlyTect BCD Plus was developed to address this need.

- Designed as a urine-based triage support test for patients presenting with hematuria or undergoing surveillance for recurrence
- Focused on enhanced sensitivity for BC, including early stage Ta and CIS cancers

EarlyTect Bladder Cancer Detection (BCD) Plus : Urine-based Dual-site PENK Methylation Test



Extracted DNA underwent bisulfite conversion and was processed using linear target enrichment (LTE) quantitative methylation-specific PCR (qMSP). This high-sensitivity technique quantified DNA methylation levels at two distinct CpG-rich regions within the *PENK* gene, with positive results defined by values exceeding predefined cut-offs at either site.

Study Design

Urine samples were prospectively collected through multicentre clinical studies in Korea and the United States. A total of 1,036 urine specimens were retrospectively analysed, comprising 892 patients with hematuria and 144 patients in post-treatment surveillance for BC recurrence

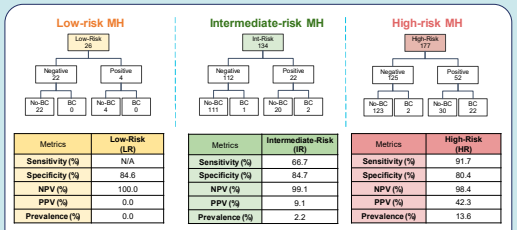
Hematuria subjects:
The study population included individuals 40 years or older who were scheduled or planned to undergo cystoscopy owing to the microscopic or gross hematuria within 3 months between March 11, 2022, and May 30, 2024. We excluded individuals who had a history of bladder cancer or upper tract urothelial cancer;were currently menstruating or had their last menstrual period within the past 3 days; had undergone invasive procedures involving the urinary tract system within the past 3 months; had suspected upper urothelial cancer lesions on ultrasonography or computed tomography; had previously received pelvic radiation therapy; had been diagnosed with other cancers and received or currently receiving chemotherapy or immunotherapy within the last 6 months; required treatment for an active urinary tract infection or vaginitis; and had undergone prostate cancer treatment or required a prostate biopsy.

Surveillance subjects:
Patients with a history of bladder cancer who were scheduled for cystoscopic surveillance following transurethral resection of bladder tumor (TURBT) were enrolled between January and December 2022. Follow-up data were collected for up to 16.5 months after the first post-TURBT visit. The risk groups were classified according to the National Comprehensive Cancer Network (NCCN) classification

Clinical Characteristics for Hematuria Patients

Characteristics	Pooled cohort, *n* (%)	
	BC (*n* = 155)	No-BC (*n* = 737)
Sex, No. (%)		
Male	130 (83.9)	402 (54.5)
Female	25 (16.1)	335 (45.5)
Age, mean (range)	69.0 (42 to 91)	65.2 (31 to 96)
Hematuria, No. (%)		
Microscopic	27 (17.4)	339 (46.0)
Gross	128 (82.6)	381 (51.7)
Unknown	-	17 (2.3)
Smoking history, No. (%)		
Never	63 (40.6)	429 (58.2)
Former	52 (33.5)	136 (18.5)
Current	36 (23.2)	77 (10.4)
Unknown	4 (2.6)	95 (12.9)

Performance based on MH Risk Stratification



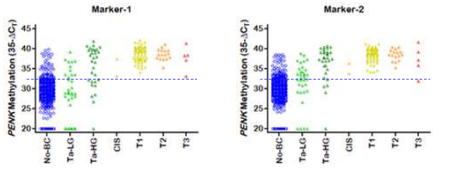
Metrics	Low-Risk (LR)
Sensitivity (%)	N/A
Specificity (%)	84.6
NPV (%)	100.0
PPV (%)	0.0
Prevalence (%)	0.0

Metrics	Intermediate-Risk (IR)
Sensitivity (%)	66.7
Specificity (%)	84.7
NPV (%)	99.1
PPV (%)	9.1
Prevalence (%)	2.2

Metrics	High-Risk (HR)
Sensitivity (%)	91.7
Specificity (%)	80.4
NPV (%)	98.4
PPV (%)	42.3
Prevalence (%)	13.6

The subjects in Korean cohort were grouped into low-, intermediate (Int)-, and high-risk categories based on risk factors such as sex, age, RBC count, and smoking history. The test results of the BCD Plus test, categorized as negative (-) or positive (+), were compared with clinical diagnoses. This analysis was restricted to the Korean cohort due to a lack of data on smoking history and RBC counts in US cohort.

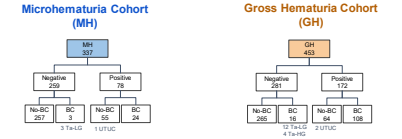
Performance for Hematuria



Category	Result
Overall Sensitivity	87.7% (81.5% – 92.5%)
Overall Specificity	82.5% (79.6% – 85.2%)
Sensitivity – Clinically Significant Tumors (Ta-HG, CIS, T1–T4)	96.6% (91.5% - 99.1%)
Sensitivity – Ta-LG	60.5% (43.4% - 76.0%)

Clinical validation of EarlyTect BCD Plus. PENK methylation status according to the tumor stage depicted as scatter plots of 35-ΔCT values in the 892 urine samples evaluated

Performance in MH vs. GH



Metric	Micro Hematuria
Sensitivity (%)	88.9
Specificity (%)	82.6
NPV (%)	98.8
PPV (%)	30.4
Prevalence (%)	8.0

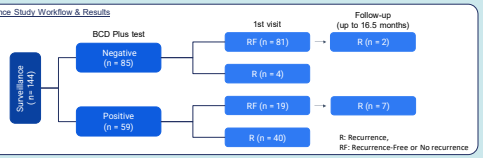
Metric	Gross Hematuria
Sensitivity (%)	87.1
Specificity (%)	80.6
NPV (%)	94.3
PPV (%)	62.8
Prevalence	27.4

The subjects in Korean cohort were grouped into microhematuria (MH) and gross hematuria (GH) categories based on RBC count. The test results of the BCD Plus test, categorized as negative (-) or positive (+), were compared with clinical diagnoses. This analysis was restricted to the Korean cohort due to a lack of data on RBC counts in US cohort.

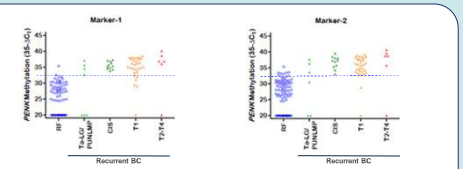
Clinical Characteristics for Surveillance Subjects

Characteristics	Surveillance Cohort, *n* (%)	
	Recurrence (*n* = 53)	Recurrence-Free (*n* = 91)
Sex, No. (%)		
Male	49 (92.5)	73 (80.2)
Female	4 (7.5)	18 (19.8)
Age, mean (range)	69.8 (46 to 86)	69.1 (47 to 86)
Previous Diagnosis, No. (%)		
NMIBC Low Risk	4 (7.5)	12 (13.2)
NMIBC Intermediate Risk	7 (13.2)	11 (12.1)
NMIBC High Risk	33 (62.3)	56 (61.5)
MIBC	9 (16.9)	12 (13.2)
Progression, No. (%)	9 (17.0)	-

Surveillance Study Workflow & Results



Performance in Surveillance



Category	Result
Overall Sensitivity	88.7% (77.0% – 95.7%)
Overall Specificity	86.8% (78.1% – 93.0%)
Sensitivity – Recurrent high-grade tumors (CIS, T1-T4)	93.6% (82.5% - 98.7%)
NPV	92.9% (86.1% - 96.6%)
PPV	79.7% (69.6% - 87.0%)

Clinical validation of EarlyTect BCD Plus. PENK methylation status according to the tumor stage depicted as scatter plots of 35-ΔCT values in the 144 urine samples evaluated. RF:Recurrence-Free or no recurrence

Summary & Conclusion

Robust Diagnostic Performance

- Combines strengths of the original EarlyTect BCD with enhanced biomarker performance
- Demonstrates strong performance in both primary and recurrent bladder cancer (BC)
- Provides a more precise and effective triage tool within established risk-stratification frameworks
- Supports clinical decision-making during initial hematuria evaluation and surveillance

Clinical Advantages in Hematuria (Initial diagnosis)

- **High Sensitivity & High NPV**: Effectively rules out BC in low-risk and intermediate-risk hematuria patients
  - Overall sensitivity was 87.7%.
  - NPV was 98.8% (100% in LR, 99.1% in IR, and 98.4% in HR patients).
  - Safely avoid 76.6% of cystoscopies for all microhematuria patients and 83.6% for intermediate-risk patients based on BCD Plus results
- **High Specificity & High PPV**: Helps resolve diagnostic uncertainty and identify high-risk patients
  - Overall specificity was 82.5%.
  - The PPV was 9.1% in IR and 42.3% in HR microhematuria.
  - The PPV was 62.8% in gross hematuria.
- **Rule-out & Rule-In**: Streamlines decision-making by helping ensure clinically significant bladder cancers are not overlooked

Clinical Advantages in Surveillance

- Urine-based non-invasive surveillance
- Potential earlier detection of high-risk BC
  - Sensitivity and specificity were 88.7% and 86.8%, respectively.
  - Sensitivity for recurrent high-grade Ta and higher-stage tumors reached 93.6%.
  - NPV and PPV in the surveillance setting were 92.9% and 79.7%.
- Prognostic and predictive value
  - Patients with positive mePENK results had a significantly higher risk of tumor recurrence.

Conclusion

- The assay demonstrates robust diagnostic performance for both primary and recurrent BC.
- Its dual-value proposition (rule-out and rule-in capability) enables:
  - Reduction of unnecessary cystoscopies in low-risk patients
  - Improved identification of high-risk patients requiring prompt evaluation
  - Reliable detection of clinically significant recurrences during surveillance

Therefore, EarlyTect BCD Plus may serve as a precise and effective triage tool within established risk-stratification frameworks.

Publications

1. CE-IVDR 2026 Registration No.: HX 2176150-1
2. Oh TJ, et al. EarlyTect BCD Plus: A urine-based dual site PENK methylation test for risk-based cystoscopy triage in hematuria. BJUI Compass 2026 Accepted for publication
3. Jeong IG, et al. Urinary DNA Methylation Test for Bladder Cancer Diagnosis. JAMA Oncol. 2025 Mar 1;11(3):293-299. PMID: 39883469
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5. Bang BR, et al. EarlyTect BCD, a Streamlined PENK Methylation Test in Urine DNA, Effectively Detects Bladder Cancer in Patients with Hematuria. J Mol Diagn. 2024 Jul;26(7):613-623. PMID: 38677548.
6. Han H, et al. Clinical Validation of the Proenkephalin (*PENK*) Methylation Urine Test for Monitoring Recurrence of Non-muscle-invasive Bladder Cancer. Eur Urol Open Sci. 2024 Mar 7;62:99-106. PMID: 38496823
7. Oh TJ, et al. Evaluation of Sensitive Urine DNA-Based PENK Methylation Test for Detecting Bladder Cancer in Patients with Hematuria. J Mol Diagn. 2023 Sep;25(9):646-654. PMID: 37330048.
8. Oh TJ, et al. Identification and validation of methylated PENK gene for early detection of bladder cancer using urine DNA. BMC Cancer. 2022 Nov 19;22(1):1195. PMID: 36403035

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