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North American study and meta-analysis evaluating performance of Bladder EpiCheck®, a FDA cleared test, in non-muscle invasive bladder cancer recurrence

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Abstract

Background: Bladder EpiCheck (BE) is a novel methylation-based PCR urine test for the detection of non-muscle invasive bladder cancer (NMIBC) recurrences.

Objective: We present the results of a North American study evaluating BE and meta-analysis of literature.

Methods: A prospective, blinded, multicenter study was conducted in North America. Voided urine was collected from NMIBC patients prior to cystoscopic surveillance. BE testing was performed centrally. For the meta-analysis, a PUBMED search was performed to identify all published peer-reviewed clinical studies of BE for NMIBC surveillance.

Results: In this study, 674 patients were enrolled of which 449 were included. Overall sensitivity was 67% (95%CI 58%-74%), specificity was 84% (80%-88%), PPV was 65% (57%-73%) and NPV was 85% (81%-89%). For high-grade (HG) recurrence, sensitivity was 77% (65%-85%) and NPV was 95% (92%-97%).

In patients with negative cystoscopy and cytology at the first study visit, risk of subsequent recurrence in 12 months was 5.3 (2.7–10.3) times higher in patients with positive BE vs. negative BE ($p < 0.0001$). In patients with negative cystoscopy and equivocal cytology, BE was positive in 75–89% of those with later HG recurrence, with PPV of 42% (15%-72%)-63% (38%-84%).

The meta-analysis included 7 studies and 1564 patients. Overall sensitivity was 82% (66–92%), HG sensitivity was 91% (82–95%), specificity was 85% (80–88%), PPV was 60% (55–64%) and HG NPV was 98% (97–99%).

Conclusions: The consistently strong performance of BE indicate that a positive test could improve timely disease recurrence detection and a negative test could rule-out HG disease. Furthermore, the low rate of false positive results, potentially minimizes unnecessary downstream procedures and patient anxiety.

Keywords

non-muscle invasive bladder neoplasms [C04.588.945.947.960.500], non-muscle invasive bladder neoplasms [C12.900.820.945.500], urine [A12.207.927], neoplasm recurrence, local [C04.697.655], real-Time polymerase chain reaction [E05.393.620.500.706], biomarkers, tumor [D23.101.140]

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Introduction

Bladder cancer is the 6th most common cancer in the United States and 4th most common cancer in men.[1,2] Recurrence rates of non-muscle invasive bladder cancer (NMIBC) depend on stage and grade and other tumor factors (multifocality, prior recurrence, carcinoma in situ), and can be as high as 30–70%, requiring close surveillance of patients with cystoscopy and urine cytology.[2] Cystoscopy is associated with minor risks such as urinary tract infection, bleeding, and pain and can be costly.[3] Cystoscopy is imperfect, and carcinoma in situ (CIS) and small tumors can be missed.[4] Furthermore, it can be difficult to distinguish between CIS and benign inflammation – which can result from intravesical therapy, prior instrumentation, and infection.[5] Cytology is an adjunct biomarker useful for identifying recurrences in patients undergoing cystoscopy with a history of high-grade (HG) tumors. Cytology has a poor sensitivity for low-grade (LG) tumors and 50–80% sensitivity for HG tumors.[6] There is also interobserver variability between pathologists assessing cytology specimens, and indeterminate results like "atypical" and "suspicious" can leave clinicians and patients confused. Many studies have evaluated urine-based tumor markers to improve the surveillance and detection of bladder cancer.[7] While urine-markers have not yet demonstrated the ability to replace cystoscopy, they have been recommended in the AUA/SUO guidelines for various clinical scenarios, such as adjudication of equivocal cytology, and to assist with evaluating response to BCG therapy.[2] The main hurdles to urine biomarker adoption into the standard-of-care are accuracy, reproducibility, and clinical utility[8] and lack of randomized studies demonstrating effective use of markers. Different clinical utility scenarios could be proposed according to each test's characteristics, for example, a rule-in test would require high specificity and a rule-out test for HG tumors would require high HG sensitivity.

Bladder EpiCheck (BE) is an objective novel methylation-based PCR urine test for detection of bladder cancer recurrence that demonstrated overall sensitivity of 68%, HG sensitivity of 89% and specificity of 88%.[9]

This manuscript presents the results of a prospective multicenter cohort study conducted to evaluate the performance of BE in patients with NMIBC undergoing surveillance in North America. Additionally, we summarize herein the body of evidence from the literature that led to BE's inclusion as an option in the EAU NMIBC guidelines.[10]

Materials and methods

A prospective, blinded, multicenter, cohort study was conducted at 11 centers in USA and Canada (NCT02700464). The primary endpoint of the study was to determine the sensitivity and specificity of the BE test as compared to the gold standard (GS) defined by cystoscopy, cytology and, if indicated, pathology. Secondary endpoints were non-inferiority of BE sensitivity and specificity vs. UroVysion's (matched and all samples) in the baseline visit (T0). Men and women aged 22 or older undergoing cystoscopic surveillance for NMIBC were enrolled within 12 months of their last TURBT. Patients planned for cystectomy or chemoradiation or those unable to give a urine sample of 45 mL, were excluded. Patients were managed according to the standard of care with cystoscopy, cytology and pathology performed locally by each site, and were followed up for up to 3 consecutive surveillance visits (Baseline – T0, 1st Follow-up visit – T1, 2nd follow-up visit – T2). Collection of urine prior to cystoscopy at each visit was used for cytology (T0, T1 and T2), BE testing (T0, T1 and T2) and UroVysion testing (T0). Physicians and patients were blinded to the results of BE, and the personnel in the lab performing BE testing was blinded to patient data, including disease status.

Study enrollment in each site started after local or central ethics committee approval was granted and all patients provided written informed consent prior to any study procedures.

Bladder EpiCheck urine test

At each visit, voided urine was collected prior to cystoscopy and sent for cytology and BE evaluation. Barbotage urine samples were excluded to align with the FDA intended use of voided urine. BE detects methylation patterns in urine cell DNA associated with urothelial carcinoma. The test requires at least 10 mL of urine. Urine is kept in refrigeration (2–8°C) for up to 5 days of collection, and then centrifuged to generate a cell pellet. DNA is extracted from the pellet using the BE kit (Nucleix Ltd., Israel) and digested using methylation sensitive enzymes provided in the BE kit. The digested DNA runs with proprietary primers and probes for 15 methylation loci, supplied in the BE kit, on the ABI® 7500 Fast Dx PCR. The minimal amount of DNA required is 5 ng per sample. The PCR output is analyzed by the BE proprietary software that calculates an EpiScore based on the detected methylation levels and reports a qualitative positive/negative result (Figure S1). BE Kit includes several controls: two run controls (No Template Control [NTC] and Undigested Control [UC]) for DNA contamination, function of PCR mixes components, and instrument detectors; and three internal sample controls (Digestion Control [DC], Digestion Control Reference [DCR], and Internal Reference [IR]) for quality of the sample (e.g., sufficient amount of DNA) and successful digestion. In case of an issue, the software will give an invalid result.

For this study, two central CLIA-certified laboratories in the U.S. were used for BE testing.

Diagnostic gold standards

The GS was considered positive if any of the following was present: (a) a positive cystoscopy confirmed by a biopsy

showing urothelial carcinoma, (b) a urine cytology positive for high-grade urothelial carcinoma (HGUC), or (c) a urine cytology suspicious for high-grade urothelial carcinoma (SHGUC). All other results were considered GS negative.

Statistical analysis

The analyses were performed on all eligible patients who were enrolled in the study and had at least one visit with all data available for GS determination and BE test result. Sensitivity, specificity, positive and negative predictive values are presented along with exact two-sided 95% confidence intervals (CI). Analyses were performed using the SAS® version 9.4 (SAS Institute, Cary NC, USA) software. Nominal p-values and non-adjusted CI are presented.

For the evaluation of the primary endpoint, one visit was included in the analysis for each patient, comparing the BE result of the urine collected on that visit to the GS of that visit. GS status and availability of a valid BE result were used to define which of the visits was selected for the analysis: the first GS positive visit with a valid BE result was used, and if all visits were GS negative, the last GS negative visit with a valid BE result was used. A patient with a GS positive visit completed the study at that visit. Statistical assumptions, including sample size calculations are detailed in the supplement.

To determine if BE could be used to anticipate future bladder cancer recurrences in patients with negative GS evaluations (i.e., negative cystoscopy and negative urine cytology), we evaluated patients whose first visit cystoscopy and cytology were negative, and had at least one more visit where cystoscopy, cytology, and BE were performed. Outcomes were compared between patients with a positive BE vs. negative BE on the first visit. Cox models were constructed to estimate the probability of a future positive GS with the BE test result as the sole covariate.

Cumulative overall sensitivity and specificity estimates calculated performance of all valid visits together (T0, T1 and T2). In this calculation a subject may appear more than one time. The calculation was done using generalized estimating equations (GEE) with Subject ID used as a repeated factor. This model accounts for potential dependency between multiple measurements within a subject. The confidence intervals are calculated using Clopper-Pearson score method.

The meta-analysis followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. A PUBMED search was performed on March 28, 2024 to identify all published peer-reviewed clinical studies of BE in NMIBC surveillance. The term "Bladder EpiCheck" was searched with no time limitations. All studies reporting original BE performance in NMIBC surveillance were included in the analysis: prospective or retrospective studies reporting true positive (TP), true negative (TN), false positive (FP), false negative (FN), and/or sensitivity and specificity. In case of not reporting TP, TN, FP, or FN, these were calculated from known variables (sensitivity and specificity) or by correspondence with the authors. Reviews, meta-analyses, case reports, modelling papers, comments, and papers not reporting the required information to calculate TN/TP/FN/FP were excluded. The most up-to-date publication was used for centers that reported on the same patients more than once. Forest plots show the results of the individual studies in the meta-analysis with 95% Two-Sided Clopper-Pearson Confidence Intervals. Meta Analysis Estimates Based on Generalized Linear Mixed Model with Paper Random Effect were calculated for the pooled performance measures.

Chi-squared test for heterogeneity was performed for these measures using the R package mada (Meta-Analysis of Diagnostic Accuracy).

Results

In total, 674 patients were prospectively enrolled from August 31 2016 until March 28 2019 of which 449 were included in this analysis, 138 patients were excluded due to barbotage urine collection method on the visit selected for analysis (Figure 1). Patient demographics and disease history are shown in Tables 1 and S1. Median age was 71 (range 37–93) and 81% were male, ethnicity, race and sex distributions were similar to those reported for the Medicare population in the USA. 47% of the patients had history of recurrence, 33% and 64% had history of T1/Tis and HG in the last recurrence, respectively, and 62% were treated with instillations since last TURBT. One-hundred thirty-one (29%) patients completed the study at T0, 75 (17%) at T1 and 243 (54%) at T2. The median time in the study was 182 (range 0–903) days.

One-hundred thirty-eight (138, 31%) patients experienced a recurrence, 64 (14%) HG, 43 (10%) LG and 31 (69%) cytology positive. Overall sensitivity was 67% (CI 95%: 58%-74%), specificity was 84% (80%-88%), PPV was 65% (57%-73%) and NPV was 85% (81%-89%). In the subpopulation with HG recurrence, sensitivity was 77% (65%-85%) and NPV was 95% (92%-97%) (Table 2). Performance was statistically different by grade but was not impacted by sex, smoking history, occupational risk or time from the last intravesical instillation treatment. In a post-hoc analysis assessing the cumulative performance of all valid visits' samples (n = 898), the prevalence was 15%, overall sensitivity was 67% (58%-74%), HG sensitivity was 77% (65%-85%), specificity was 81% (77%-84%), PPV was 42% (35%-48%), overall NPV was 93% (90%-95%) and HG NPV was 98% (96%-99%).

There was evidence that BE could anticipate future bladder cancer recurrences in patients with negative

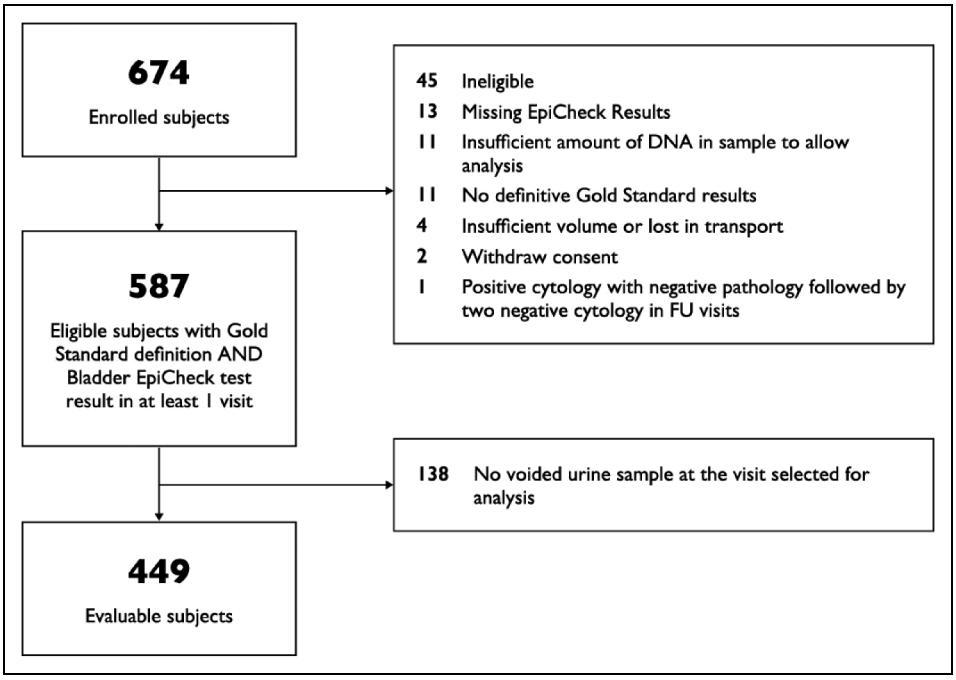

Figure 1. CONSORT table of the North American clinical study.

cystoscopy and cytology since the risk of any recurrence and HG recurrence in 12 months was 5.3 (HR = 5.3 [2.7-10.3], P < 0.0001) and 5.1 (HR = 5.1 [1.5–17.5], p = 0.0106) times higher, respectively, in BE positive patients (n = 56) compared to BE negative patients (n = 228)(Figures 2(a) and (b)). The PPV for such patients was 38% (n/N = 21/56, 25%-52%) and the median time from a positive BE test result to GS conversion to positive was 182 days.

There were 53, 40, and 21 equivocal visits (visit with a negative cystoscopy and atypical urothelial cells (AUC) result in cytology) at T0, T1 and T2, respectively, all were without biopsy and were considered GS negative at that visit (Table 3). BE was positive in 19 (36%), 12 (30%) and 6 (29%) of equivocal visits, respectively. In patients with at least one visit after the equivocal visit, 16 (30%) of T0 and 6 (15%) of T1 became GS positive in a later visit. BE was already positive at the equivocal visit in 12 (75% [48%-93%]) and 5 (83% [36%-99.6%]), respectively, of the patients who eventually converted to GS positive. Nine and 4 of the T0 and T1 visits, respectively, became HG in a later visit of which 8 (89% [52%-99.7%]) and 3 (75% [19%-99.4%]) were already positive by BE at the equivocal visit. BE PPV was 63% (38%-84%) and 42% (15%-72%) for later recurrence, respectively.

To compare BE and cytology performance, patients with both valid BE and a cytology result on the selected visit were compared to pathologically confirmed cancers. Overall sensitivity was 62% (59%-75%) for BE and 53% (44%-61%) for cytology. HG sensitivity in this sub-analysis was 73% (59%-85%) and 43% (29%-58%), respectively. BE specificity was 84% (80%-88%), however, cytology specificity could not be calculated, because all cytology HGUC and SHGUC were considered as GS positive in our analysis.

BE demonstrated non-inferiority to UroVysion (data not shown).

In the meta-analysis, of the 25 BE peer-reviewed articles appearing in the search, 7 unique clinical studies on NMIBC surveillance were identified,[9,12–17] including a sponsored prospective multicenter European clinical study and 6 independent studies (Figure 3). In a pooled analysis 1564 patients with NMIBC undergoing surveillance were included, 357 had an event (241 HG and 116 LG). BE demonstrated an overall sensitivity of 82% (66–92%), HG sensitivity of 91% (82–95%) and specificity of 85% (80–88%) (Table 4 and Figures 4(a), (b), and (c)). PPV was 60% (55–64%) and HG NPV was 98% (97–99%). Chi-squared test for equality of overall sensitivity and specificity rejected the null hypothesis of equality ($p < 0.001$ and $p = 0.0326$, respectively) and the Chi-squared test for equality of HG sensitivity did not reject the null hypothesis of equality ($p = 0.197$). Three of the studies focused on high-risk patients or patients with history of HG,[13,14,17] driving the overall sensitivity up. When these studies are excluded from the pooled analysis, overall sensitivity is lower at 73% (57%-85%), and overall specificity is similar at 85% (79%-89%) (Figures 4(d) and (e)). Results were similar between

Table 1. Patient demographics in the North American clinical study.

Demographics	Included in analysis	Medicare US bladder cancer patients 2021 (prevalence)[11]
N	449	730,044
Age, y Median (range)	71 (37–93)	NR
Sex	(N/%)	(N/%)
Male	365 (81%)	551,797 (76%)
Female	84 (19%)	178,247 (24%)
Ethnicity, N		574,406
Not Hispanic or Latino	431 (96%)	NR
Hispanic or Latino	15 (3%)	23,898 (4%)
Not Reported	3 (1%)	–
Race		
White	411 (92%)	525,695 (92%)^
Black	19 (4%)	27,211 (5%)[#]
Asian	11 (2%)	12,192 (2%)^
American Indian or Alaska Native	3 (0.7%)	826 (0.1%)^
Not Reported	5 (1%)	–
Disease History	**N (%)**	
History of Recurrence		
Primary	239 (53%)	
Recurrent	210 (47%)	
Stage in last recurrence		
Ta	301 (67%)	
T1	88 (20%)	
Tis	60 (13%)	
CIS present	95 (21%)	
Grade in last recurrence		
Low Grade/PUNLMP	163 (36%)	
High Grade	286 (64%)	
Instillation Treatment prior to enrollment		
<90 days from last instillation to first visit	201 (45%)	
>90 days from last instillation to first visit	74 (16%)	
Unknown time from last instillation to first visit	3 (1%)	
No Instillation Treatment prior to enrollment	171 (38%)	

^Non-Hispanic; [#]Including Hispanic.

Table 2. Bladder EpiCheck performance in the North American clinical study.

Overall performance	n/N	% (95% CI)	
Sensitivity	92/138	67% (58%-74%)	
Specificity	262/311	84% (80%-88%)	
PPV	92/141	65% (57%-73%)	
NPV	262/308	85% (81%-89%)	
Performance by grade	**n/N**	**Sensitivity**	**NPV**
High Grade	49/64	77% (65%-85%)	95% (92%-97%)
Low Grade	20/43	47% (33%-61%)	93% (89%-95%)
Positive by cytology	23/31	74% (57%-86%)	97% (95%-99%)

PPV, positive predictive value; NPV, negative predictive value.

the North American clinical study and the pooled analysis, with overlapping CI95% for overall sensitivity 67% (58%-74%) vs. 73% (57%-85% [only mixed studies]), specificity 84% (80%-88%) vs. 85% (80–88% [all studies])/85% (79%-89% [only mixed studies]) and HG sensitivity 77% (65%-85%) vs. 91% (82–95%), respectively.

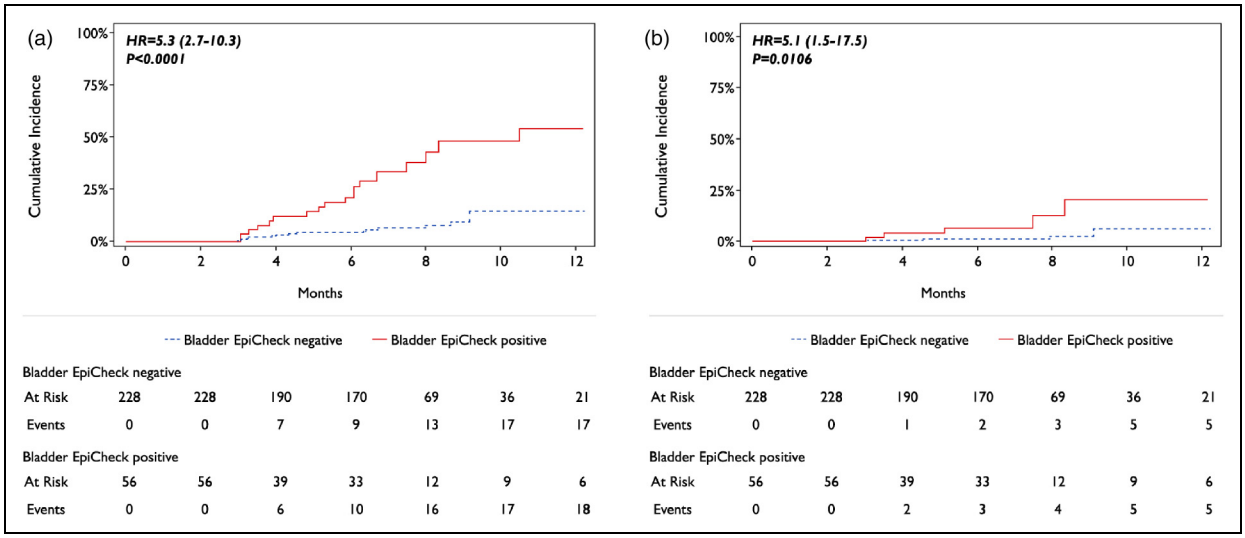

Figure 2. Anticipatory positive. Solid line represents the cumulative incidence of a recurrence in patients with a positive Bladder EpiCheck result and negative gold standard (cystoscopy and cytology) result at T0. Dotted Line represents the cumulative incidence of a recurrence in patients with a negative Bladder EpiCheck result and negative gold standard (cystoscopy and cytology) result at T0. (a) Time from T0 with Negative Cystoscopy and Cytology to Any Recurrence at Later Visits. (b). Time from T0 with Negative Cystoscopy and Cytology to High-Grade Recurrence at Later Visits.

Table 3. Equivocal visits – patients with a negative cystoscopy and equivocal cytology in the North American clinical study.

	Number of subjects with an equivocal visit*	Bladder EpiCheck positive	Later Gold Standard positive	Bladder EpiCheck anticipatory positive	Later high-grade	Bladder EpiCheck high-grade anticipatory positive	Bladder EpiCheck PPV for later recurrences (CI95%)
T0	53	19	16	12	9	8	63% (38%-84%)
T1	40	12	6	5	4	3	42% (15%-72%)
T2	21	6	N/A	N/A	N/A	N/A	N/A

* Visit was defined as equivocal if cystoscopy was negative and cytology result was atypical urothelial cells (AUC).
PPV, positive predictive value; N/A, not applicable.

Table 5 details sensitivity by stage in the North American clinical study, the European multicenter study and pooled results from all peer-reviewed publications reporting results by stages.

Discussion

This North American clinical study demonstrated BE overall sensitivity of 67%, HG sensitivity of 77% and specificity of 84%. In the cumulative analysis, specificity was slightly lower (81%) compared to the longitudinal analysis, but overall and HG sensitivity were identical, due to study design (patients with a GS positive completed the study, so the number of positive events was the same in the two analyses). The main difference between the longitudinal and cumulative results are PPV and NPV, since these measures are impacted by disease prevalence which was 15% in the cumulative analysis vs. 31% in the longitudinal analysis. PPV, NPV and HG NPV were 65%, 85%, 95% for the longitudinal analysis and 42%, 93% and 98% for the cumulative analysis, respectively. The cumulative predictive values probably better represent the per-visit predictive values of the test, as they do not inflate disease prevalence beyond a single visit. The findings in this study are consistent with the meta-analysis which included the European multicenter clinical study.[9] In the pooled analysis of 1564 patients with NMIBC under surveillance, the pooled HG sensitivity and HG NPV were 91% and 98%, respectively. Equally important was the high specificity of 84% in this clinical study which was corroborated in the meta-analysis with pooled specificity of 85%. One main criticism of urine markers is the low specificity resulting in low overall PPV. With a high PPV, BE provides a benefit by raising the specter of missed malignancy suggesting the need for further evaluation with biopsies, enhanced cystoscopy or meticulous surveillance monitoring. One could also consider upper tract imaging if there has not been any recent evaluation. High specificity is very important in clinical decision-making to avoid dealing with false positive results, since every 5% drop in specificity could translate to 30% more false positive cases. There were only 3 MIBC recurrences in the study, of which

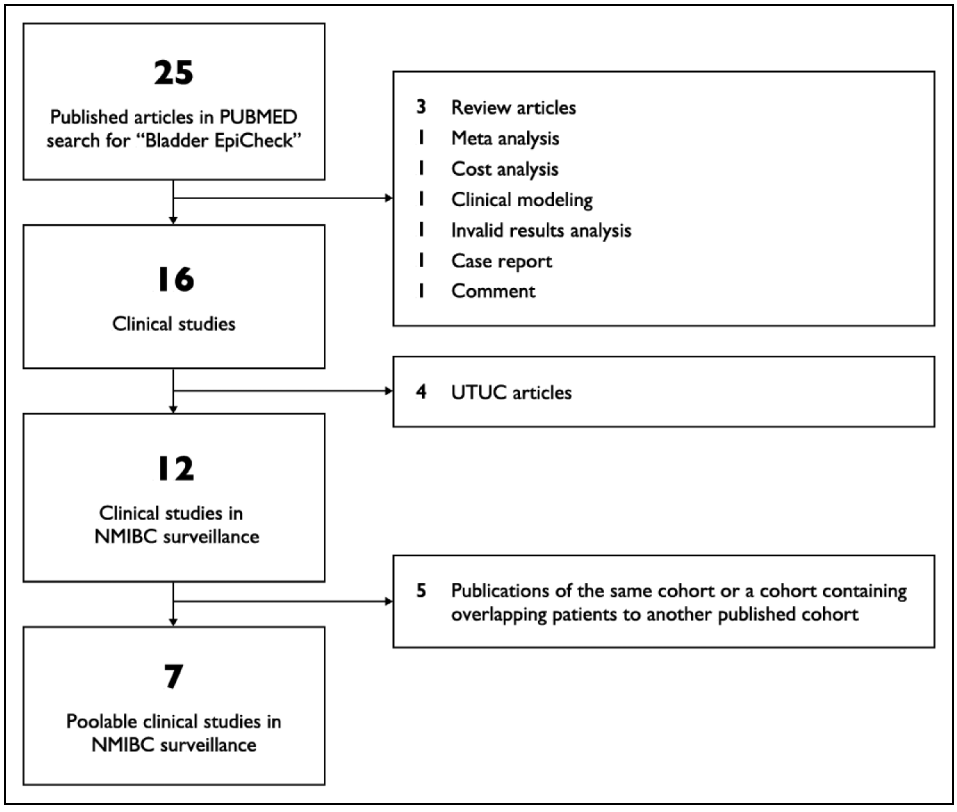

Figure 3. Literature review for Bladder EpiCheck evidence.

2 were missed by the test. It is not possible to assess the performance of the test in such a small sample size and a larger study in such patients would be required.

The AUA/SUO guidelines currently identify a role for urine markers to help adjudicate patients with equivocal cytology.[2] In this prospective North American clinical study, BE identified at the equivocal visit 75–89% of later detected HG disease. The evaluation of equivocal cytology could be aided by BE because a negative test indicates the probability of CIS is low. Furthermore, the high PPV for future recurrences in this population (42–63%), reinforces its utility in this setting.

Multiple reports have shown the potential for "anticipatory findings" with urine markers, apparently false positive results that actually indicate an early or undetected tumor. In this study, patients with negative cystoscopy and cytology who had a positive BE result at their first study visit had a ~5 times higher risk of any/HG recurrence, compared to those with a negative BE result. This suggests that BE's specificity may be higher than the reported 84%, since 38% of the apparent false positive results of the test are actually false negative results of both cystoscopy and cytology. With such significant risk of recurrence, a positive BE test in the face of a negative cystoscopy could warrant further evaluation with more sensitive tools, such as enhanced cystoscopy, to detect recurrence 6 months earlier, or help guide intensification of surveillance for patients who are on long intervals between cystoscopies. An earlier detection could potentially identify BCG non-responders, as recommended in the AUA/SUO guidelines for older urine markers. For example, detecting CIS prior to development of T1, or detecting T1 prior to the development of muscle invasive disease, allowing for earlier intervention with potentially improved oncological outcomes.

The study's robust eligibility, keeping inclusion broad with minimal exclusion criteria, resulted in a study population representative of the USA bladder cancer population including age, sex, race, and ethnicity and had good balance between patients with and without a history of recurrence, history of HG and LG as well as patients with and without recent instillations.

One of the concerns with urine marker studies is the lack of independent validation. The finding of similar performance in the North American study results and the meta-analysis supports the robustness of BE and the generalizability of results across diverse populations. The fact that BE testing was performed in local labs with a commercially distributed FDA cleared kit and still had similar performance to a central lab supports the ability to use it on a widespread basis.

BE's specificity was very similar across publications and among the published papers. Such robust results can be explained by the very limited susceptibility of the test to interference by common urinary conditions and urine constituents, such as infection, inflammation, hematuria, and recent instillation treatments demonstrated in analytical

Table 4. Meta-analysis performance of Bladder EpiCheck in NMIBC surveillance.

Published clinical studies	Study design	company sponsored	Study population	Included in the pooled analysis?	number of evaluable patients	Recurrence (N)	disease prevalence	HG recurrence (HG N)	HG disease prevalence	no cancer	BE true positive (n)	BE HG true positive (HG n)	BE true negative	sensitivity	HG sensitivity	specificity	NPV	HG NPV	PPV
Witjes et al. Eur Urol Oncol. 2018[9]	**Pr, Mc, Sv**	**yes**	**NMIBC surveillance**	**yes**	**353**	**44**	**12%**	**18**	**5%**	**309**	**30**	**16**	**272**	**68%**	**89%**	**88%**	**95%**	**99%**	**45%**
D'Andrea et al. BJU Int. 2019[18]	Pr, Mc, Sv	yes	NMIBC surveillance	no – same cohort as Witjes et al.	357	49	14%	18	5%	308	33	16	271	67%	89%	88%	94%	99%	47%
Trenti et al. Cancer Cytopathol. 2020[12]	**Pr, Sc, Sv**	**no**	**NMIBC surveillance**	**yes**	**432**	**92**	**21%**	**38**	**9%**	**340**	**59**	**30**	**279**	**64%**	**79%**	**82%**	**89%**	**98%**	**49%**
Trenti et al. Cancer Cytopathol. 2019[19]	Pr, Sc, Sv	no	NMIBC surveillance	no – same patients as Trenti et al. 2020	215	69	32%	30	14%	146	43	25	126	62%	83%	86%	83%	97%	68%
Pierconti et al. Urol Oncol. 2021[13]	**Re, Sc, 1y FU**	**no**	**HG NMIBC surveillance with instillations**	**yes**	**375**	**111**	**30%**	**111**	**30%**	**264**	**104**	**104**	**208**	**94%**	**94%**	**79%**	**97%**	**97%**	**65%**
Pierconti et al. J Clin Pathol. 2021[20]	Pr, Sc, Sv	no	NMIBC surveillance	no – same patients as Pierconti et al. 2021	374	127	34%	127	34%	247	121	121	209	95%	95%	85%	97%	98%	76%
Pierconti et al. Urol Oncol. 2022[21]	Pr, Sc, Sv	no	HG NMIBC surveillance with instillations	no – same patients as Pierconti et al. 2021	205	151	74%	151	74%	54	142	142	43	94%	94%	80%	83%	86%	93%
Cochetti et al. Urol Oncol. 2022[14]	**Pr, Sc, Sv**	**no**	**High Risk NMIBC PDD surveillance**	**yes**	**40**	**18**	**45%**	**8**	**20%**	**22**	**18**	**8**	**20**	**100%**	**100%**	**91%**	**100%**	**100%**	**90%**
Peña et al. J Clin Med. 2022[15]	**Pr, Sc, Sv**	**no**	**NMIBC surveillance**	**yes**	**57**	**13**	**23%**	**12**	**21%**	**44**	**12**	**12**	**36**	**92%**	**100%**	**82%**	**97%**	**100%**	**60%**
Ragonese et al. Clin Genitourin Cancer. 2022[16]	**Pr, Sc, Sv**	**no**	**NMIBC surveillance**	**yes**	**209**	**71**	**34%**	**53**	**25%**	**138**	**57**	**48**	**117**	**80%**	**91%**	**85%**	**89%**	**97%**	**73%**
Pierconti et al. Cancer Cytopathol. 2023[22]	Re, Sc, 1y FU	no	High Risk NMIBC surveillance	no – same patients as Pierconti et al. 2021	290	127	44%	127	44%	163	127	127	115	100%	100%	71%	100%	100%	73%
Caño Velasco et al. Actas Urol Esp. 2023[17]	**Pr, Sc, Sv**	**no**	**High Risk NMIBC surveillance**	**yes**	**98**	**8**	**8%**	**1**	**1%**	**90**	**7**	**1**	**82**	**88%**	**100%**	**91%**	**99%**	**100%**	**47%**
Pooled analysis (only unique studies appearing in bold)					**1564**	**357**	**23%**	**241**	**17%**	**1207**	**287**	**15%**	**1014**	**82%**	**91%**	**85%**	**94%**	**98%**	**60%**

BE, Bladder EpiCheck; FU, follow-up; HG, high-grade; Mc, multicenter; NMIBC, non-muscle invasive bladder cancer; NPV, negative predictive value; PDD, Photodynamic diagnosis; PPV, positive predictive value; Pr, prospective; Re, retrospective; Sc, single center; Sv, single visit.

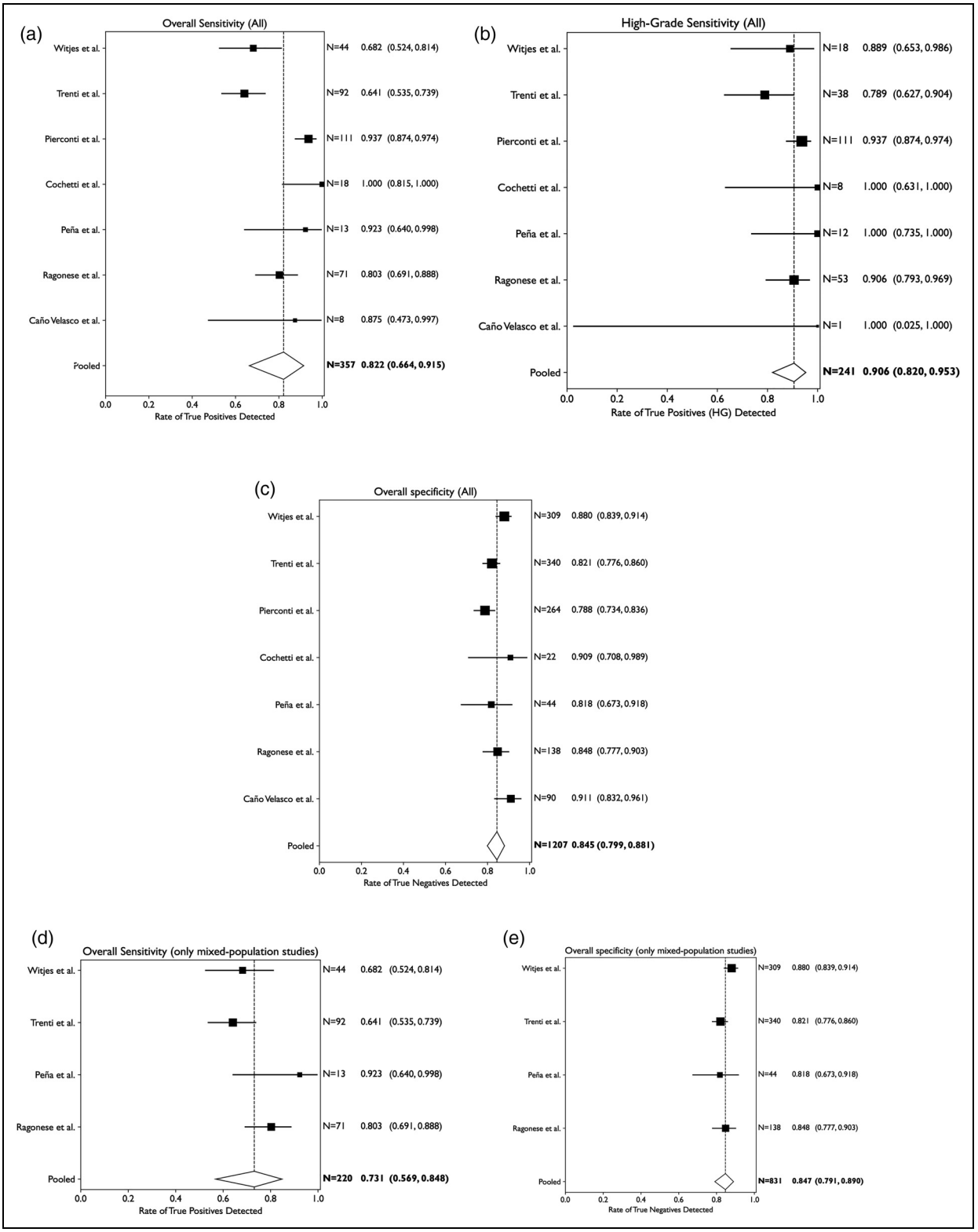

Figure 4. Meta-analysis forest plots. (a) Overall Sensitivity (All). (b) High-Grade Sensitivity. (c) Overall Specificity (All). (d) Overall Sensitivity (Only studies with mixed population). (e) Overall Specificity (Only studies with mixed population).

Table 5. Bladder EpiCheck sensitivity by stage.

	North American Clinical study		European Study		Literature (pooled, including European study)	
Stage	n/N	% (95% CI)	n/N	% (95% CI)	Number of studies reporting	% (n/N)
Ta	38/65	59% (46%-70%)	14/27	52% (32–71)	2[9,14]	67% (26/39)
T1	7/11	64% (35%-85%)	7/7	100% (59–100)	3[9,14,17]	100% (9/9)
T2	1/3	33% (6%-79%)	NR	NR	1[14]	100% (1/1)
CIS^	10/13	77% (50%-92%)	3/3	100% (29–100)	3[9,14,16]	90% (28/31)

^Studies did not differentiate between concomitant or stand-alone CIS.
NR, not reported.

interference studies,[23] the North American clinical study, and the European multicenter clinical study.[9]

The main limitation to this analysis was the exclusion of 138 patients due to collection of barbotage urine instead of voided urine. Importantly, omitting these patients from the analysis did not bias the population demographics and disease history (Table S1). Only 1.6% of patients were excluded from the analysis due to insufficient DNA. An additional 2% were excluded from the analysis due to missing BE result for other reasons (e.g., failure of internal control). The results were robust and consistent between the North American clinical study and the prior studies in the literature.

The results of randomized controlled-trials, such as the German UroFollow study[24] and the ongoing American "Replace Cysto" study,[25] are needed to guide use of BE in clinical practice.

Conclusions

The consistently strong performance of BE indicate that the test could have a potential utility both as a rule-in as well as a HG rule-out test. A strong anticipatory positive signal could translate into earlier detection of recurrence, potentially leading to better outcomes. The test could help adjudicate equivocal cytology and identify BCG-non responders, as currently recommended in the AUA/SUO guidelines for older urine markers. A negative test could be used to rule-out HG disease, and identify patients who could delay a cystoscopy. Furthermore, the low rate of false positive results, potentially minimizes unnecessary downstream procedures and patient anxiety.

Randomized studies will help to expand our understanding of use cases and provide additional evidence for test use.

Statements and declarations

Author contributions

Author	conception	performance of work	interpretation of data	writing the article
Neil Fleshner		X	X	X
H. Barton Grossman	X		X	X
Ryan Berglund		X		
Jason Hafron		X		X
Brant Inman		X	X	X
Lawrence Karsh		X		
Kelvin Moses		X		
Daniel Saltzstein		X		
Anup Shah		X		
Jonathan Wright		X		
J Alfred Witjes				X
Yair Lotan			X	X

Declaration of conflicting interests

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Data availability

North American study - the data supporting the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Meta-analysis - the data supporting the findings of this study are available within the article and/or its supplementary material.

Supplemental material

Supplemental material for this article is available online.

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