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Diagnostic Performance of Urinary Keratin 17 Immunocytochemistry (URO17®) During Hematuria Evaluation: A Retrospective Study

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Abstract

Background/Objectives: Urinary biomarkers may assist hematuria risk stratification, but diagnostic performance must be assessed against an appropriate reference standard. We evaluated urinary keratin 17 immunocytochemistry (URO17®) during hematuria evaluation and compared it with urine cytology. **Methods:** This retrospective, single-center study included adults evaluated between August 2019 and March 2026. The analytic cohort comprised 746 specimens from 612 patients. Formal diagnostic accuracy analyses used one result-independently selected specimen per patient in the reference-standard cohort. URO17® positivity was defined as at least 10 keratin 17-positive cells. Sensitivity and specificity were compared using paired McNemar tests; atypical cytology was classified as negative in the primary comparison. **Results:** The reference-standard analysis included 313 patients, including 20 with histopathologically confirmed urothelial carcinoma. URO17® sensitivity was 90.0% (95% confidence interval, 69.9–97.2), specificity 90.1% (86.1–93.0), and negative predictive value 99.2% (97.3–99.8). Cytology sensitivity was 20.0% and specificity 98.6%. URO17® was 70.0 percentage points more sensitive and 8.5 percentage points less specific (both $p < 0.001$). Quantitative cell counts yielded an area under the receiver operating characteristic curve of 0.938. Patients without reference-standard verification were analyzed descriptively. **Conclusions:** URO17® demonstrated higher sensitivity but lower specificity than conventional cytology when atypical cytology was classified as negative. The high negative predictive value reflects, in part, the 6.4% cancer prevalence in the verified cohort. Selective verification and the small number of cancer-positive patients limit interpretation. Prospective multicenter validation is needed to define its adjunctive clinical role.

Keywords: urothelial carcinoma; hematuria; keratin 17; URO17; urine cytology; diagnostic accuracy; urinary biomarker

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1. Introduction

Hematuria is a common indication for urologic evaluation because it may reflect malignancy, urinary stone disease, infection, or other benign conditions [1]. Contemporary evaluation commonly includes cystoscopy and upper urinary tract imaging. Urothelial carcinoma (UC) remains a principal diagnostic concern, although its prevalence among patients presenting with hematuria is relatively low. In a population-based study of 134,173 patients

with hematuria, cumulative risks of invasive bladder cancer were approximately 1.9% within 3 months and 3.31% at 5 years following hematuria diagnosis in men [2]. Consequently, many patients undergo invasive evaluation without a subsequent malignancy diagnosis.

Recent work has emphasized combining clinical risk assessment with diagnostic testing. A national imaging cohort reported a low overall yield of upper tract findings, supporting risk-based imaging selection [3]. Studies incorporating the Hematuria Cancer Risk Score with ultrasound and decision-curve-based strategies have also examined how clinical characteristics might individualize cystoscopy decisions [4,5]. These approaches provide context for evaluating whether urinary biomarkers add information beyond established risk factors.

Cystoscopy remains the reference standard for evaluation of the lower urinary tract [1]. Urine cytology is used as a noninvasive adjunct but has limited sensitivity, particularly for low-grade disease [6], and atypical findings may create diagnostic uncertainty [7]. These limitations have contributed to interest in urinary biomarkers for noninvasive UC risk stratification [8]. Current AUA/SUFU guidelines recognize that appropriately selected urinary biomarkers may inform decisions regarding cystoscopy in counseled patients with intermediate-risk microscopic hematuria [1].

Recent biomarker studies have evaluated mRNA expression, genomic alterations, DNA methylation, and urinary proteins in hematuria populations [9–13]. A genomic assay has also been evaluated within AUA hematuria risk categories [14]. These platforms differ in biological targets, patient selection, and intended use; performance estimates from one assay cannot be assumed to apply to another.

URO17® is a urine-based immunocytochemical assay designed to detect keratin 17 (K17)-positive urothelial cells. K17 has been identified as a biomarker of urothelial neoplasia [15], and urine-based K17 immunocytochemical testing has demonstrated favorable diagnostic performance for UC detection [16]. Recent K17 studies include a hematuria cohort, procedure-enriched populations, and laboratory comparisons with cytology and fluorescence in situ hybridization [16–18]. Tissue-based work has also examined K17 in upper tract UC, although tissue immunohistochemistry does not establish the accuracy of a voided-urine assay [19]. We evaluated URO17® during microscopic (MH) and gross hematuria (GH) evaluation in a community urology practice. The intended clinical role evaluated here is as a pre-cystoscopy adjunct for risk stratification during hematuria evaluation that may inform subsequent diagnostic decision-making, rather than as a replacement for cystoscopy, imaging, or histopathologic assessment or as a substitute for guideline-directed evaluation when clinically indicated. An exploratory analysis evaluated patients with atypical urine cytology. The contribution of the present study is a paired, patient-level comparison in patients without prior UC presenting for hematuria evaluation, using a fixed ≥10-cell cutoff and separating reference-standard verification from descriptive clinical follow-up.

2. Materials and Methods

2.1. Study Design and Participants

This retrospective diagnostic accuracy study used routinely collected clinical records from adults undergoing hematuria evaluation at Urology Associates of Silicon Valley (San Jose, CA, USA) between August 2019 and March 2026. Eligible patients were ≥18 years of age, had no prior UC, and provided a voided urine specimen for URO17® testing. Exclusion criteria were prior UC or cystectomy, non-voided specimen type, indeterminate URO17® result, or insufficient clinical information for outcome classification. No minimum follow-up duration was required; however, the clinical follow-up cohort required at least one completed assessment sufficient for retrospective outcome classification. Follow-up duration was measured from URO17® specimen collection to the last subsequently documented

completed clinical encounter or diagnostic assessment. The source population comprised all patients who underwent URO17® testing at this practice during the study period; the analytic cohort was obtained after applying the eligibility and specimen exclusions described above. Testing, further diagnostic evaluation, and follow-up were provided as part of routine clinical care. There was no study-mandated diagnostic pathway, randomized allocation, or standardized schedule of investigations.

Among 883 assessed specimens, 26 (2.9%) had an indeterminate URO17® result and were excluded. An additional 107 non-voided specimens and 4 specimens with insufficient clinical information were excluded. No specimens were excluded because of a missing URO17® result. An indeterminate URO17® result was defined as a tested specimen for which a definitive positive or negative classification could not be issued because of insufficient cellularity, poor staining quality, or another technical limitation affecting interpretation. Figure 1 summarizes specimen inclusion and exclusion.

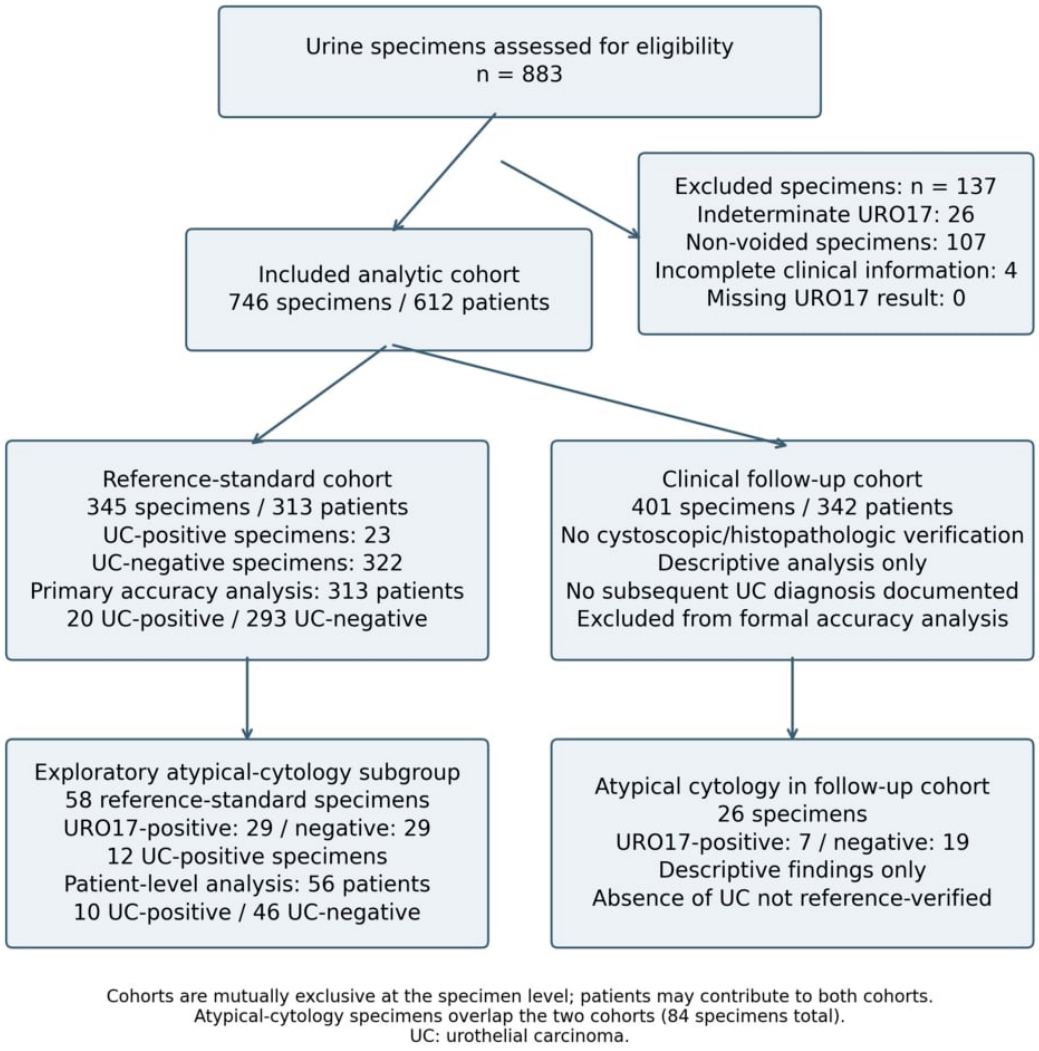


Figure 1. Specimen eligibility, reference-standard verification, and exploratory atypical-cytology analyses. Formal accuracy analyses used one eligible specimen per patient within the reference-standard cohort; clinical follow-up specimens were analyzed descriptively. Patients may contribute specimens to both cohorts, but each specimen belongs to only one cohort.

The study was approved by WCG Institutional Review Board (IRB No. 20262349), with informed consent waived because of the retrospective design. Reporting followed STARD 2015 [20].

2.2. Reference Standard and Outcome Verification

Specimens were assigned to a reference-standard cohort or clinical follow-up cohort according to the available diagnostic evaluation. The reference-standard cohort included patients with documented cystoscopic and/or histopathologic evaluation. UC-positive outcomes required histopathologic confirmation. UC-negative outcomes were established by a nonconcerning cystoscopic evaluation; imaging and subsequent clinical follow-up, when available, were considered supportive information but were not used in place of cystoscopic reference-standard verification. The clinical follow-up cohort included specimens without documented cystoscopic or histopathologic reference-standard evaluation. Outcome assessment in this cohort incorporated imaging, cytology, urinalysis, urine culture, repeat urine testing, and subsequent urologic encounters. Because verification intensity and follow-up were nonstandardized, the clinical follow-up cohort was analyzed descriptively and excluded from all formal diagnostic accuracy calculations. Decisions regarding cystoscopy versus clinical follow-up were made by treating urologists using their assessment of patient risk and the available initial findings, including age, smoking history, imaging, urine cytology, and URO17® results. In routine practice, patients considered at lower risk with reassuring initial findings were generally managed with repeat urine assessment and clinical follow-up, whereas concerning findings prompted consideration of further evaluation, including cystoscopy. This describes the practice's clinical approach rather than a uniform study-defined decision rule. Cohort assignment reflected the diagnostic evaluation documented in the records; patient-specific reasons for each verification pathway were not determined in this analysis.

URO17® results were available in the electronic medical record to treating urologists and were considered alongside other clinical findings when selecting subsequent diagnostic evaluation. Cystoscopists could have had access to URO17® results, whereas pathologists interpreting histopathology were blinded to URO17® results. Characteristics of the reference-standard and clinical follow-up cohorts were compared descriptively to assess potential selective verification (Table S1). The URO17® index date was the specimen collection date. Reference-standard timing was calculated from URO17® collection to the corresponding cystoscopic evaluation or, when histopathology established UC, the diagnostic biopsy. For patients diagnosed with UC, follow-up was censored at the initial histopathologic diagnosis; subsequent surveillance evaluations were excluded.

2.3. Index Test

Voided urine specimens were collected as part of hematuria evaluation and processed at Clinical Laboratory Improvement Amendments–certified laboratories using standardized immunocytochemical methods for K17 detection. Slides were evaluated by pathologists or trained laboratory personnel blinded to clinical findings and reference-standard outcomes. Results were quantified by the number of K17-positive urothelial cells per slide and dichotomized using a fixed threshold of ≥10 cells.

Earlier published urine-based K17 work used a 5-cell positivity threshold [18]. Homayoun et al. evaluated candidate thresholds of ≥5, ≥10, ≥15, and ≥20 cells in the same underlying cohort of 746 specimens from 612 patients at the same center during August 2019–March 2026 [21]. In that specimen-level analysis, ≥10 cells was the highest evaluated candidate threshold maintaining sensitivity above 90%, with fewer false-positive classifications than ≥5 cells. The present study applies this fixed cutoff to a patient-level, reference-standard-verified subset and compares URO17® with paired cytology. This is a reanalysis of a shared dataset, not independent external validation of the cutoff; the cutoff is not characterized here as prospectively prespecified.

2.4. Statistical Analysis

Formal diagnostic accuracy analyses were restricted to the reference-standard cohort and conducted at the patient level. For patients with multiple eligible specimens, the first eligible specimen was selected using a result-independent rule. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR−) were calculated for URO17® at ≥10 K17-positive cells. Sensitivity, specificity, PPV, and NPV were reported with 95% Wilson confidence intervals and likelihood ratios with logarithmic 95% confidence intervals.

Urine cytology was evaluated in the same patient-level population. High-grade urothelial carcinoma (HGUC) and suspicious for high-grade urothelial carcinoma (SHGUC) were classified as positive; negative and atypical results were classified as negative. Differences in sensitivity and specificity were reported with 95% confidence intervals estimated using 10,000 patient-level bootstrap resamples and compared using two-sided McNemar tests. A sensitivity analysis alternatively classified atypical cytology as positive. The atypical-cytology subgroup was evaluated as exploratory. Additional analyses by hematuria presentation and sex were likewise restricted to the reference-standard cohort and considered descriptive because of the limited numbers of UC-positive patients within individual strata (Table S2). ROC analysis of quantitative K17-positive cell counts was performed within the patient-level reference-standard cohort, with AUC reported with a 95% confidence interval.

No formal prospective sample-size calculation was performed; all eligible patients meeting predefined criteria during the study period were included. No minimum acceptable sensitivity or maximum false-negative rate was prespecified for the present retrospective analysis. Analyses were performed in R version 4.4.0 using complete-case data. Tests were two-sided with $p < 0.05$ considered statistically significant.

3. Results

3.1. Study Cohort and Verification

The analytic cohort included 746 specimens from 612 patients. Median age was 68 years (Q1–Q3, 57–77), 432 patients (70.6%) were male, and 171 (28.0%) were current or former smokers (Table 1). At the specimen level, 316 specimens (42.4%) were obtained during gross hematuria evaluation and 430 (57.6%) during microscopic hematuria evaluation; 23 specimens (3.1%) were UC-positive and 84 (11.3%) had atypical cytology. The reference-standard cohort comprised 345 specimens from 313 unique patients and included all 23 UC-positive specimens; 20 unique patients with UC were included in the primary patient-level analysis. The reference-standard cohort included higher proportions of patients with gross hematuria, URO17®-positive results, and atypical cytology than the clinical follow-up cohort. Because patients could contribute different specimens to each verification category, 43 patients contributed to both cohorts (313 + 342 − 612). Thus, 299 patients had no specimen included in the reference-standard cohort; the 342 patients in the clinical follow-up cohort should not be interpreted as a mutually exclusive group of unverified patients.

The median interval from URO17® collection to reference-standard evaluation was 21 days (Q1–Q3, 0–46; range, 0–1121 days). Reference-standard evaluation occurred on the same day in 82 patients (26.2%) and after URO17® collection in 231 (73.8%); 272 patients (86.9%) underwent reference-standard evaluation within 90 days. The clinical follow-up cohort comprised 401 specimens from 342 unique patients. Median specimen-level documented follow-up was 2.53 months (Q1–Q3, 0.39–16.06; range, 0–70.80 months). Of 401 specimens, 168 (41.9%) had at least 6 months of follow-up, including 119 (29.7%) with at least 12 months; 168 (41.9%) had greater than 0 but less than 6 months of follow-up, and 65 (16.2%) had same-day evaluation only (Table S3).

Table 1. Baseline Patient Characteristics.

Characteristic	Value
Patient-level characteristics	
Patients, *n*	612
Age, median (Q1–Q3), y	68 (57–77)
Sex, *n* (%)	
Female	180 (29.4)
Male	432 (70.6)
Race, *n* (%)	
White	390 (63.7)
Asian	95 (15.5)
Black or African American	10 (1.6)
Other	37 (6.1)
Unknown	63 (10.3)
Patient refused	17 (2.8)
Ethnicity, *n* (%)	
Not Hispanic or Latino	519 (84.8)
Hispanic or Latino	49 (8.0)
Patient refused	17 (2.8)
Unknown	27 (4.4)
Smoking status, *n* (%)	
Non-smoker	437 (71.4)
Former smoker	148 (24.2)
Current smoker	23 (3.8)
Unknown	4 (0.6)
Specimen-level characteristics	
Specimens, *n*	746
Hematuria type, *n* (%)	
Gross hematuria	316 (42.4)
Microscopic hematuria	430 (57.6)
UC-positive specimens, *n* (%)	23 (3.1)
Atypical cytology specimens, *n* (%)	84 (11.3)

Demographic and smoking characteristics are patient-level; hematuria type, UC status, and cytology are specimen-level.

3.2. Patient-Level Diagnostic Performance

The primary patient-level reference-standard analysis included 313 patients, including 20 (6.4%) with histopathologically confirmed UC. URO17® yielded 18 true-positive, 2 false-negative, 29 false-positive, and 264 true-negative results. Urine cytology yielded 4 true-positive, 16 false-negative, 4 false-positive, and 289 true-negative results when atypical cytology was classified as negative (Table 2). URO17® sensitivity was 90.0% (95% CI, 69.9–97.2), specificity 90.1% (95% CI, 86.1–93.0), PPV 38.3% (95% CI, 25.8–52.6), NPV 99.2% (95% CI, 97.3–99.8), LR+ 9.09 (95% CI, 6.25–13.23), and LR− 0.11 (95% CI, 0.03–0.41).

Cytology sensitivity was 20.0% (95% CI, 8.1–41.6), specificity 98.6% (95% CI, 96.5–99.5), PPV 50.0% (95% CI, 21.5–78.5), and NPV 94.8% (95% CI, 91.6–96.7). The paired sensitivity difference was +70.0 percentage points (95% CI, 50.0–90.0; McNemar $p < 0.001$), and the specificity difference was −8.5 percentage points (95% CI, −11.9 to −5.5; McNemar $p < 0.001$).

Table 2. Patient-Level Diagnostic Performance of URO17® in the Reference-Standard Cohort.

Measure	URO17®	Urine Cytology	Paired Comparison
Patients, *n*	313	313	–
UC-positive patients, *n*	20	20	–
True positive, *n*	18	4	–
False negative, *n*	2	16	–
False positive, *n*	29	4	–
True negative, *n*	264	289	–
Sensitivity, % (95% CI)	90.0 (69.9–97.2)	20.0 (8.1–41.6)	–
Specificity, % (95% CI)	90.1 (86.1–93.0)	98.6 (96.5–99.5)	–
PPV, % (95% CI)	38.3 (25.8–52.6)	50.0 (21.5–78.5)	–
NPV, % (95% CI)	99.2 (97.3–99.8)	94.8 (91.6–96.7)	–
LR+ (95% CI)	9.09 (6.25–13.23)	14.65 (3.95–54.29)	–
LR− (95% CI)	0.11 (0.03–0.41)	0.81 (0.65–1.01)	–
Sensitivity difference, percentage points (95% CI)	–	–	+70.0 (50.0–90.0)
McNemar *p* value for sensitivity	–	–	<0.001
Specificity difference, percentage points (95% CI)	–	–	−8.5 (−11.9 to −5.5)
McNemar *p* value for specificity	–	–	<0.001

Analyses included 313 patients in the reference-standard cohort. URO17® positivity was defined as ≥10 K17-positive urothelial cells. For the primary cytology analysis, HGUC and SHGUC were positive; negative and atypical cytology were negative. Differences are URO17® minus cytology.

In the sensitivity analysis classifying atypical cytology as positive, cytology yielded 14 true-positive, 6 false-negative, 48 false-positive, and 245 true-negative results, corresponding to 70.0% sensitivity (95% CI, 48.1–85.5) and 83.6% specificity (95% CI, 78.9–87.4). The paired differences for URO17® versus cytology were +20.0 percentage points for sensitivity (95% CI, 5.0–40.0; McNemar $p = 0.046$) and +6.5 percentage points for specificity (95% CI, 2.4–10.6; McNemar $p = 0.002$). The two URO17® false-negative UC specimens contained 5 and 7 K17-positive cells; one was low-grade UC with stage unavailable and the other was high-grade pTa UC (Table S4).

3.3. Quantitative Discrimination

Within the patient-level reference-standard cohort, quantitative URO17® cell counts yielded an AUC of 0.938 (95% CI, 0.911–0.964) (Figure 2). The primary comparison with urine cytology was based on paired diagnostic performance at the respective clinical thresholds rather than ROC-curve comparison because URO17® cell counts are quantitative whereas urine cytology is categorical.

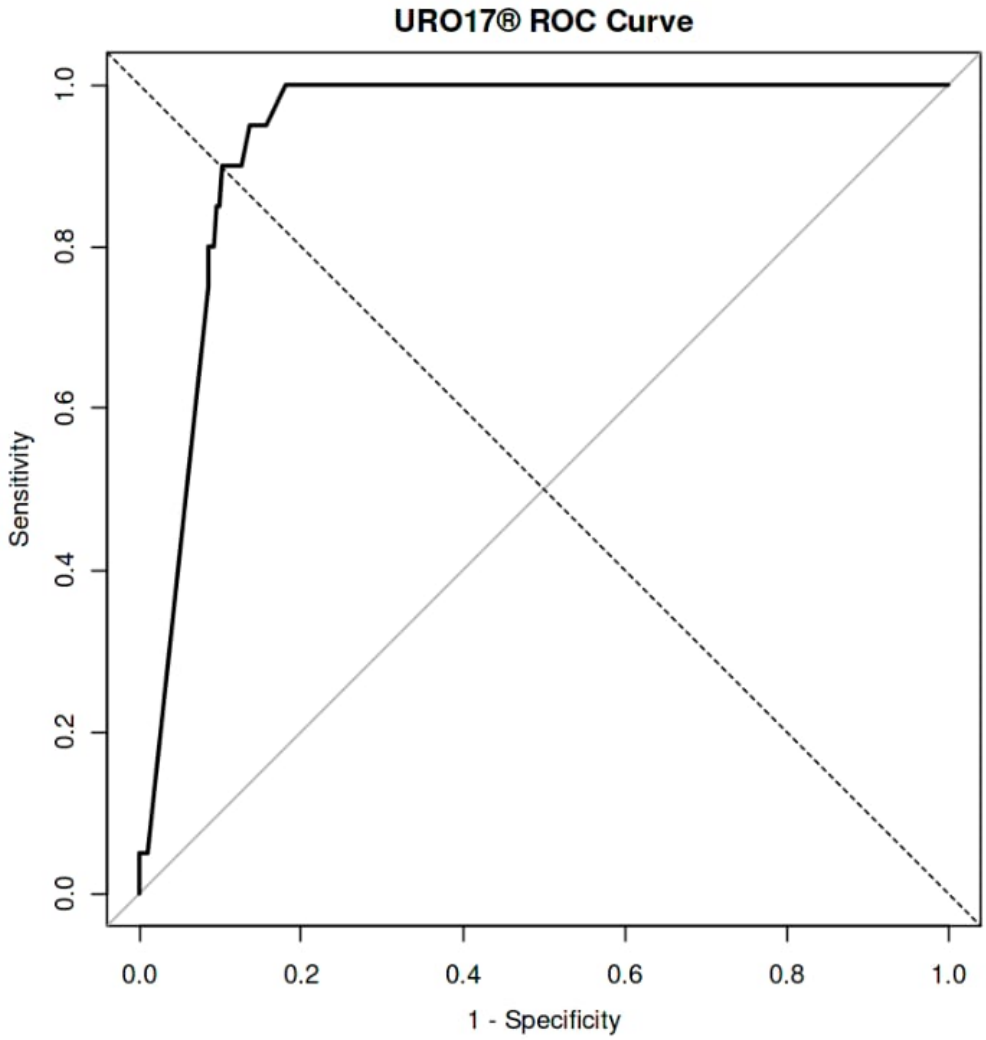


Figure 2. Receiver operating characteristic curve. Quantitative URO17® cell counts in the patient-level reference-standard cohort (n = 313) yielded an AUC of 0.938 (95% CI, 0.911–0.964). The solid black line represents the URO17® ROC curve. The ascending light gray diagonal represents chance-level discrimination (AUC = 0.5). The descending dashed diagonal marks points where sensitivity equals specificity.

3.4. Exploratory Atypical-Cytology Analysis

The exploratory atypical-cytology subgroup comprised 84 specimens: 58 in the reference-standard cohort and 26 in the clinical follow-up cohort. Within the reference-standard cohort, URO17® yielded 12 true-positive, 0 false-negative, 17 false-positive, and 29 true-negative results at the specimen level; thus, 29 specimens were URO17® positive and 29 were URO17® negative. Within the clinical follow-up cohort, 7 specimens were URO17® positive and 19 were URO17® negative. The patient-level reference-standard analysis included 56 patients with atypical cytology, including 10 with UC. URO17® yielded 10 true-positive, 0 false-negative, 17 false-positive, and 29 true-negative results, corresponding to 100.0% sensitivity (95% CI, 72.2–100.0), 63.0% specificity (95% CI, 48.6–75.5), 37.0% PPV (95% CI, 21.5–55.8), and 100.0% NPV (95% CI, 88.3–100.0) (Table 3). At the specimen level, 12 of 58 reference-standard atypical-cytology specimens were associated with UC, all of which were URO17® positive; these represented 10 unique UC-positive patients.

Table 3. Patient-Level URO17® Performance Among Patients with Atypical Cytology in the Reference-Standard Cohort.

Measure	URO17®
Patients, *n*	56
UC-positive patients, *n*	10
True positive, *n*	10
False negative, *n*	0
False positive, *n*	17
True negative, *n*	29
Sensitivity, % (95% CI)	100.0 (72.2–100.0)
Specificity, % (95% CI)	63.0 (48.6–75.5)
PPV, % (95% CI)	37.0 (21.5–55.8)
NPV, % (95% CI)	100.0 (88.3–100.0)

Analysis was restricted to patients with atypical cytology who underwent reference-standard evaluation and used one eligible specimen per patient.

4. Discussion

In the patient-level reference-standard cohort, URO17® demonstrated 90.0% sensitivity (95% CI, 69.9–97.2), 90.1% specificity (95% CI, 86.1–93.0), and 99.2% NPV (95% CI, 97.3–99.8) for UC detection. The LR− of 0.11 (95% CI, 0.03–0.41) further supports the potential value of a negative URO17® result in risk stratification. URO17® should be interpreted as an adjunct to clinical evaluation rather than a replacement for cystoscopy, imaging, or histopathologic assessment when indicated [1].

At their respective clinical thresholds, URO17® demonstrated greater sensitivity than urine cytology when atypical cytology was classified as negative (90.0% vs. 20.0%), whereas cytology demonstrated greater specificity (98.6% vs. 90.1%). In paired analyses, URO17® sensitivity was 70.0 percentage points higher and specificity was 8.5 percentage points lower. When atypical cytology was alternatively classified as positive, cytology sensitivity increased to 70.0% and specificity decreased to 83.6%; under this definition, URO17® remained 20.0 percentage points more sensitive and 6.5 percentage points more specific. These findings illustrate the effect of how atypical cytology is classified and support comparison of the tests at clinically defined thresholds rather than emphasizing differences in ROC AUC.

The clinical follow-up cohort was retained for descriptive characterization but excluded from formal diagnostic accuracy analyses because cystoscopic or histopathologic reference-standard verification was unavailable. Follow-up within this cohort was heterogeneous and frequently limited: median specimen-level documented follow-up was 2.53 months, 41.9% of specimens had at least 6 months of follow-up, and 29.7% had at least 12 months. In addition, 16.2% had an additional same-day clinical or diagnostic evaluation but no subsequently documented completed encounter on a later date. Treating urologists and cystoscopists could also have known URO17® results when subsequent evaluation was selected, introducing potential selective verification bias. Characteristics of the reference-standard and clinical follow-up cohorts are therefore reported separately in Table S1.

The atypical cytology subgroup represents a clinically relevant area of uncertainty because atypical findings may be difficult to interpret and can lead to additional testing [7,22]. In the patient-level reference-standard analysis, all 10 patients with UC were URO17® positive. At the specimen level, all 12 atypical-cytology specimens associated with UC were URO17® positive. Because the subgroup contained relatively few UC-positive patients

and the confidence intervals were wide, these findings should be considered exploratory. Analyses by hematuria presentation and sex were similarly limited by small UC-positive counts and were therefore reported descriptively without conclusions regarding comparative subgroup performance.

Recent Paris System analyses reinforce the importance of distinguishing atypical cytology from categories suspicious or positive for high-grade malignancy [23,24]. The change in cytology performance when atypical findings were classified as positive in our sensitivity analysis illustrates why the cytology definition must accompany any claim of comparative sensitivity. It does not imply that atypical cytology is clinically equivalent to a negative finding.

Urinary biomarker studies span initial diagnosis and surveillance and show substantial heterogeneity in patient selection, reference standards, and test performance [8,25–27]. Recent primary studies have evaluated Xpert mRNA testing, enhanced Cxbladder assays, and PENK methylation in hematuria populations [9–12]. Additional studies have investigated novel methylation markers and the analytical performance of Cxbladder Triage Plus [28,29]. Analytical validation establishes assay characteristics and should be distinguished from evidence that a test safely changes clinical management. These studies support continued investigation of noninvasive risk stratification but do not provide a direct head-to-head comparison with URO17®.

Earlier URO17® investigations provide useful but non-equivalent comparators. Porten et al. evaluated 152 participants undergoing urologic procedures and reported patient-level sensitivity of 90% and specificity of 88%, with substantially lower specificity within the suspected-UC subgroup [18]. Ibrahim et al. studied 98 patients presenting with hematuria, separately from 51 surveillance patients, reporting 100% sensitivity and 96.15% specificity in the hematuria group [17]. Guo et al. compared 2378 urine specimens with cytology and UroVysion FISH; this laboratory comparison differs from the cystoscopic/histopathologic verification used here [16]. The present study evaluates a community hematuria population with no prior UC, a ≥10-cell threshold rather than the earlier 5-cell criterion, and paired cytology definitions. Differences in population, threshold, unit of analysis, and verification preclude attributing between-study performance differences to assay improvement. In contrast to these external studies, the threshold-optimization report [21] used the same underlying dataset. The added contribution here is the restriction of formal accuracy estimates to verified patients, selection of one specimen per patient, and paired cytology comparisons, rather than a new cohort or independent threshold validation.

False-positive results are clinically consequential. URO17® produced 29 false-positive classifications among 293 patients without verified UC, compared with four for cytology under the primary definition. Clinical findings among false-positive URO17® specimens in the reference-standard cohort are summarized in Table S5. A positive result could prompt additional cystoscopy, repeat cytology, imaging, or biopsy, with associated discomfort, anxiety, procedural risk, and cost; these downstream outcomes were not measured. The PPV of 38.3% means that most URO17®-positive patients in this verified cohort did not have confirmed UC. Although inflamed tissue and urothelial atypia were associated with false-positive results in an earlier procedure-based study [18], those findings cannot establish the mechanism of positivity in our patients. Without adequate longitudinal verification, positive results should not be reclassified as early cancer detection.

The two false-negative URO17® results occurred in low-grade UC with unavailable stage and high-grade pTa UC; the respective specimens contained 5 and 7 K17-positive cells. These cases demonstrate that the ≥10-cell threshold can miss both low- and high-grade disease. A lower threshold would classify these two specimens as positive, but its net effect on specificity and clinical utility cannot be inferred without a full threshold analysis. With

only 20 cancer-positive patients, the 90.0% sensitivity estimate has a wide confidence interval (69.9–97.2%); precise conclusions about grade- or stage-specific sensitivity are therefore unsupported. In particular, a negative URO17® result cannot exclude high-grade UC.

A potential clinical use would be to provide additional information when a patient undergoing hematuria evaluation has atypical cytology or when a clinician is integrating clinical risk factors with other diagnostic findings. This remains a proposed application rather than a management strategy validated by this study. Illustrative URO17® case reports describe use alongside standard investigations but do not establish a safe cystoscopy-deferral pathway [30]. The STRATA randomized trial evaluated a different assay, Cxbladder Triage, and demonstrated reduced cystoscopy use in its defined lower-risk group [31]. That assay-specific clinical utility cannot be transferred to URO17®. Current AUA/SUFU guidance permits urine cytology or validated markers in appropriately counseled intermediate-risk microhematuria patients who wish to avoid cystoscopy and accept the risk of forgoing direct visualization; this does not remove the need for appropriate imaging and follow-up [1]. Prospective URO17® studies should measure incremental value over clinical risk, missed high-grade cancers, downstream procedures, patient experience, and costs before defining such a pathway.

This study has several limitations. Its retrospective, single-center design may limit generalizability. Diagnostic evaluation was physician-directed, and treating urologists had access to URO17® results, and URO17® was one component of clinical decision-making. Consequently, selection for reference-standard verification was related to clinical risk and potentially to the index-test result, creating selective verification bias; restricting formal accuracy estimates to verified patients does not eliminate this bias. The clinical follow-up cohort had heterogeneous and frequently limited longitudinal follow-up and therefore could not reliably establish absence of UC; accordingly, this cohort was excluded from formal diagnostic accuracy calculations. Although the primary analysis was conducted at the patient level to avoid within-patient correlation from repeated specimens, only 20 unique patients had UC, resulting in wide confidence intervals around sensitivity and limiting precision in subgroup analyses. Finally, this diagnostic accuracy study cannot determine whether incorporating URO17® into hematuria evaluation reduces cystoscopy use, improves clinical outcomes, or is cost-effective. The ≥10-cell cutoff was evaluated previously in this same underlying dataset [21], so the present estimates may retain optimism associated with data-informed threshold selection and require independent validation.

The high negative predictive value should be interpreted in the context of the 6.4% cancer prevalence in the verified patient cohort. Predictive values will change with disease prevalence and referral setting; a negative result does not establish the safety of omitting otherwise indicated evaluation. The exploratory atypical-cytology findings require independent confirmation. Because care was delivered in routine practice, the timing and completion of diagnostic evaluation and follow-up were not standardized and depended on clinical decisions and patient attendance. The study period also overlapped the COVID-19 pandemic, although its contribution to individual intervals was not quantified. Prolonged intervals may weaken the correspondence between disease status at urine collection and at verification, while short or incomplete follow-up may miss subsequent cancer. These factors may introduce temporal misclassification and selective ascertainment. Although 272 of 313 patients (86.9%) were verified within 90 days, a time-restricted accuracy analysis was not performed.

5. Conclusions

In the patient-level reference-standard cohort, URO17® demonstrated 90.0% sensitivity and 90.1% specificity for UC detection. Its high NPV should be interpreted in the context of

the 6.4% cancer prevalence and selective verification. URO17® was more sensitive but less specific than conventional cytology when atypical cytology was classified as negative; a high-grade pTa cancer was among the false-negative results. These findings support further study of URO17® as an adjunct to standard evaluation. Prospective multicenter studies with consistent verification and adequate follow-up are needed to define clinical utility and patient selection.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/diagnostics16223846/s1, Table S1. Comparison of Reference-Standard and Clinical Follow-up Cohorts. Table S2. URO17® Diagnostic Performance and Urothelial Carcinoma Prevalence by Hematuria Type and Sex. Table S3. Duration of Documented Follow-Up in the Clinical Follow-Up Cohort. Table S4. Tumor Grade and Stage Among All UC-Positive Specimens. Table S5. Clinical Findings Among False-Positive URO17® Specimens in the Specimen-Level Reference-Standard Cohort.

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Conflicts of Interest: S.S.G. serves as Chief Medical Officer of KDx Diagnostics. S.J. is Co-Founder, President, and Chief Operating Officer of KDx Diagnostics. N.W.K. is Co-Founder, Chief Executive Officer, and Chief Technology Officer of KDx Diagnostics. S.H. and C.J. are independent contractors for KDx Diagnostics. Y.L. serves as a consultant for KDx Diagnostics and holds stock options in the company. A.B., D. Nudell, D. Noller, and M.N. report no relevant conflicts of interest.

Abbreviations

AUC, area under the receiver operating characteristic curve; CI, confidence interval; GH, gross hematuria; HGUC, high-grade urothelial carcinoma; IRB, institutional review board; K17, keratin 17; LR+, positive likelihood ratio; LR−, negative likelihood ratio; MH, microscopic hematuria; NPV, negative predictive value; PPV, positive predictive value; Q1–Q3, first to third quartile; ROC, receiver operating characteristic; SHGUC, suspicious for high-grade urothelial carcinoma; STARD, Standards for Reporting Diagnostic Accuracy Studies; UC, urothelial carcinoma.

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