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A Rapid, Non-Invasive Fluorescence-Based Urinary Exfoliated Cell Assay as an Adjunctive Diagnostic Test for Urothelial Carcinoma in Patients with Hematuria: A Prospective Diagnostic Study

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Abstract

Background

Hematuria often prompts investigation for urinary malignancy, but cystoscopy is invasive and resource-dependent. We evaluated a rapid fluorescence urinary exfoliated-cell assay as an auxiliary test for urothelial carcinoma (UC) in patients with hematuria.

Methods

In this single-center prospective diagnostic study (Ruijin Hospital, April–July 2026), 94 patients were analyzed after exclusions. All underwent fluorescence staining; DNA ploidy (n = 81) and hematoxylin and eosin (H&E) staining (n = 80) were comparators. Histopathology was the reference standard. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), 95% confidence intervals (CIs), Kappa, and paired McNemar tests were calculated.

Results

There were 55 UC and 39 benign cases. Fluorescence sensitivity was 70.9% (95% CI 57.9–81.2), specificity 76.9% (61.6–87.4), PPV 81.3%, NPV 65.2%, and Kappa 0.466. DNA ploidy sensitivity was 24.5%, specificity 100%, and NPV 41.2%; H&E sensitivity was 23.1%, specificity 100%, and NPV 41.2%. Fluorescence was more sensitive than both comparators ($P < 0.001$); DNA ploidy and H&E did not differ ($P = 1.0$). Limitations include single-center design, small subgroups, 26/131 quality-control exclusions, non-random missing comparators, few upper tract cases, and an unvalidated RNA mechanism.

Conclusions

The assay is auxiliary, not a replacement. Negative fluorescence cannot exclude UC; high-risk patients need cystoscopy and upper tract evaluation. Multicenter validation is required.

Trial registration:

ClinicalTrials.gov NCT07813260, retrospectively registered on September 2026.

1 Background

Hematuria is common in urinary tract disease and an important warning sign of malignancy; approximately 10%–20% of patients with gross hematuria are ultimately diagnosed with urinary tract tumors [1]. Cystoscopy, ureteroscopy and imaging are the main diagnostic approaches, but endoscopy is

invasive, costly and often poorly accepted. The 2025 American Urological Association guideline continues to recommend cystoscopy as the main tool for bladder cancer while using risk stratification to guide decisions [2].

Conventional urinary cytology is non-invasive and specific, but its sensitivity is low, especially for low-grade tumors, at approximately 4%–31% [3]; sensitivity may be as low as 0% for low-grade lesions and approximately 36% for high-grade tumors [4]. DNA ploidy analysis detects aneuploidy by DNA image cytometry but requires dedicated equipment and remains insufficiently sensitive.

The assay evaluated here uses a tricyclic aromatic cation composite fluorescence reagent containing SYTOX Green and propidium iodide (PI). SYTOX Green mainly targets nuclear DNA and produces bright green nuclear fluorescence, showing nuclear size, morphology and chromatin density [5, 6]. PI mainly binds cytoplasmic RNA; in metabolically active abnormal cells it accumulates and produces orange-red cytoplasmic fluorescence, complementing the green nuclear signal [7]. Normal and inflammatory degenerative cells have low cytoplasmic RNA and show almost no PI orange-red fluorescence, whereas malignant urinary cells show abundant cytoplasmic RNA, clear orange-red fluorescence and nuclear abnormalities such as enlarged nuclei, increased nuclear-to-cytoplasmic ratio, irregular contours and coarse chromatin clumping [8]. This dual-fluorescence plus morphology system may help distinguish inflammatory degenerative cells from malignant cells and overcome the limited visibility of conventional H&E staining for small lesions.

International studies have supported SYTOX Green/PI dual fluorescence for cytopathological imaging and have shown differences in nuclear abnormality, metabolism and cytoplasmic RNA enrichment between urinary tumor cells and normal/inflammatory cells [5–8]. Fluorescence microscopy can display cell morphology and metabolic fluorescence and improve detection of early lesions [9–11]. Urinary fluorescence cytology has been adopted in some Western centers as a non-invasive screening and pre-cystoscopy triage tool [12]. However, many foreign products use single-dye systems and lack standardized medical-device products adapted to urinary exfoliated cells. In China, fluorescence urinary cytology has developed rapidly; domestic studies support its ability to highlight nuclear abnormalities and high metabolism, distinguish inflammatory degenerative cells from malignant cells, and address the low sensitivity and subjectivity of conventional H&E staining [13, 14]. The reagent used here has completed national Class I medical-device filing and standardized quality control. We therefore prospectively evaluated this assay as an auxiliary test in hematuria and compared it with DNA ploidy analysis and conventional H&E staining.

2 Materials and Methods

2.1 Study Design

The aim of this study was to evaluate the diagnostic performance of a rapid fluorescence urinary exfoliated-cell assay as an auxiliary test for UC in patients with hematuria. This single-center prospective

diagnostic study was conducted in the urology outpatient and inpatient departments of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine. The protocol was approved by the institutional ethics committee, and all patients provided written informed consent. The study was prospectively registered at ClinicalTrials.gov (NCT07813260).

2.2 Study Participants

From April to July 2026, 131 samples were collected from the urology outpatient and inpatient departments of Rui Jin Hospital, Shanghai Jiao Tong University School of Medicine. After quality control (QC), 26 were excluded, leaving 105. Then 5 non-UC cases (1 squamous cell carcinoma, 3 adenocarcinoma, 1 renal cell carcinoma) and 6 cases without definitive pathology were excluded. Finally, 94 patients with a definitive final diagnosis who met the inclusion/exclusion criteria were analyzed (Fig. 1).

Inclusion criteria were: hematuria requiring investigation for urinary malignancy; age ≥ 18 years; planned cystoscopy and/or ureteroscopy or surgery; ability to sign informed consent. Exclusion criteria were: previously diagnosed urinary malignancy; participation in another clinical trial within 3 months; clinical conditions judged by the investigator to interfere with study conduct; insufficient or unqualified urine sample.

2.3 Assay Methods

Fresh urine (≥ 30 mL) was collected before endoscopy or surgery. Exfoliated cells were enriched by high-speed centrifugation, fixed and prepared as smears using a standardized workflow. The tricyclic aromatic cation composite fluorescence reagent (Taizhou Pinrui Medical Technology Co., Ltd.; filing no. □□□20250099) was used for fluorescence staining, and signals including DNA abnormalities, increased cytoplasmic RNA and morphological features were read. The reagent has completed Chinese Class I medical-device filing, with standardized production, quality control and batch consistency. Regarding nomenclature, the “tricyclic aromatic cation” mainly corresponds to PI (a phenanthridine tricyclic aromatic cation structure); SYTOX Green does not have this structure.

After staining, images were observed under a blue-light excitation fluorescence microscope using standardized double-blind reading. Two trained cytopathologists independently reviewed slides without knowledge of clinical data, other assay results or final pathology; slide order was randomized. At least 50 fields were evaluated per slide. Disagreements were resolved by a third senior pathologist.

Positive criteria combined dual fluorescence and pathological nuclear abnormalities: significantly enlarged nuclei, increased nuclear-to-cytoplasmic ratio, irregular nuclear contours, coarse chromatin clumping and disordered nuclear structure; bright green/yellow-green nuclear fluorescence with orange-red cytoplasmic fluorescence. Representative images are shown in Fig. 2: (A) normal urothelial cells with bright green nuclei and gray-green cytoplasm, reflecting stable diploid DNA and low RNA metabolic activity (negative); (B) UC cells with bright yellow nuclei and orange-yellow cytoplasm, consistent with

abnormal DNA and RNA (positive, moderate); (C) UC cells with bright yellow nuclei and orange-red cytoplasm, reflecting markedly increased DNA and RNA (positive, high).

DNA ploidy and urinary exfoliated-cell H&E results were collected from the Rui Jin Hospital patient information 360 system. Some patients had undergone contrast-enhanced CT urography before enrollment and declined additional tests; DNA ploidy and/or H&E were omitted by shared decision-making, not for technical reasons. All 94 patients underwent fluorescence staining.

2.4 Gold Standard

Histopathology from biopsy specimens obtained by endoscopy or surgery was the reference standard for UC. Positive was pathologically confirmed UC. Negative was non-malignant disease, including inflammation, hyperplastic lesions, cysts and stones; benign diagnoses were based on histopathology when available or on clinical/imaging findings when tissue was not obtained.

2.5 Statistical Analysis

Sample size was estimated before initiation. Based on fluorescence sensitivity approximately 70% [11], DNA ploidy sensitivity approximately 25% [10], a paired McNemar design, two-sided $\alpha = 0.05$ and 80% power, the minimum paired positive cases were 24. Considering approximately 20% invalid results and an expected UC prevalence of 58%, at least 52 patients were required; 94 were included.

Sensitivity, specificity, PPV, NPV and 95% CIs were calculated using the Clopper–Pearson exact method. Agreement was assessed using Kappa. Analyses used SPSS version 26.0 (IBM Corp., Armonk, NY, USA). Two-sided $P < 0.05$ was significant.

2.6 Quality Control and Excluded Samples

QC was performed before slide interpretation by laboratory personnel blinded to final pathology, using pre-defined criteria: fewer than 20 urothelial cells per 10 high-power fields; blood, mucus or inflammatory exudate obscuring evaluation; transport delay > 2 hours; bacterial overgrowth. A worst-case sensitivity analysis assessed excluded samples.

3 Results

3.1 Baseline Characteristics

Of 131 samples, 26 were excluded by QC, leaving 105; 5 non-UC and 6 without definitive pathology were then excluded, leaving 94. Men were 69 (73.4%) and women 25 (26.6%). Age ranged from 21 to 87 years, median 67, mean ± SD 65.3 ± 12.5 years.

By final diagnosis, 55 (58.5%) were UC and 39 (41.5%) benign. Among 55 UC, 35 (63.6%) were high-grade and 20 (36.4%) low-grade. T stage was Ta 31 (56.4%), Tis 2 (3.6%), T1 12 (21.8%), T2 7 (12.8%), T4 1 (1.8%) and Tx 2 (3.6%). Tumor location was bladder in 49 (89.2%), renal pelvis in 2 (3.6%), ureter in 2

(3.6%), bladder plus ureter in 1 (1.8%), and renal pelvis plus ureter in 1 (1.8%) (Table 1). Percentages for final diagnosis use 94 as denominator; those for location, grade and T stage use 55 UC cases.

Table 1
Baseline Demographic and Clinicopathological Characteristics of the Study Cohort.

Age	**Total (N = 94)**
Mean ± SD	65.3 ± 12.5
Median (range)	67 (21–87)
Sex, n (%)	
Male	69 (73.4)
Female	25 (26.6)
Final diagnosis, n (%)	
Urothelial carcinoma	55 (58.5)
Non-Urothelial carcinoma	39 (41.5)
Tumor location, n (%)	
Bladder	49 (89.2)
Ureter	2 (3.6)
Renal pelvis	2 (3.6)
Ureter + Bladder	1 (1.8)
Renal pelvis + Ureter	1 (1.8)
Histological grade, n (%)	
Low-grade	20 (36.4)
High-grade	35 (63.6)
Pathological T stage, n (%)	
Ta	31 (56.4)
Tis	2 (3.6)
T1	12 (21.8)
T2	7 (12.8)
T4	1 (1.8)
Tx	2 (3.6)

3.2 Overall Diagnostic Performance of the Three Assays

Fluorescence was positive in 48, including 39 true positives and 9 false positives; 46 were negative, including 30 true negatives and 16 false negatives. Sensitivity was 70.9% (57.9–81.2), specificity 76.9% (61.6–87.4), PPV 81.3% (68.0–89.8), NPV 65.2% (49.6–78.2), and Kappa 0.466 (moderate).

DNA ploidy was definitive in 81: 13 positive, all true positives; 68 negative, including 28 true negatives and 40 false negatives. Sensitivity was 24.5% (14.9–37.6), specificity 100.0% (87.9–100.0), PPV 100.0% (77.2–100.0), NPV 41.2% (30.3–53.0), Kappa 0.184.

H&E was definitive in 80: 12 positive, all true positives; 68 negative, including 28 true negatives and 40 false negatives. Sensitivity was 23.1% (13.7–36.1), specificity 100.0% (87.9–100.0), PPV 100.0% (75.7–100.0), NPV 41.2% (30.3–53.0), Kappa 0.174 (Table 2).

Table 2
Overall Diagnostic Performance of the Three Assays.

Sensitivity (95% CI)	**Fluorescence staining**	**DNA ploidy analysis**	**H&E staining**
	70.9%	**24.5%**	**23.1%**
	(57.9%-81.2%)	**(14.9%-37.6%)**	**(13.7%-36.1%)**
Specificity (95% CI)	76.9%	100.0%	100.0%
	(61.6%-87.4%)	(87.9%-100.0%)	(87.9%-100.0%)
PPV	81.3%	100.0%	100.0%
(95% CI)	(68.0%-89.8%)	(77.2%-100.0%)	(75.7%-100.0%)
NPV	65.2%	41.2%	41.2%
(95% CI)	(49.6%–78.2%)	(30.3%-53.0%)	(30.3%-53.0%)
Kappa	0.466	0.184	0.174

3.3 Subgroup Analysis

For bladder tumors (n = 49), sensitivity was 73.5% (59.2–84.2) for fluorescence, 20.8% (11.5–34.2) for DNA ploidy and 18.8% (10.0–32.0) for H&E (Supplementary Table S1). Other location subgroups had small samples and wide CIs; results are exploratory (Supplementary Tables S2–S5).

For low-grade UC (n = 20), sensitivity was 70.0% (47.3–85.6) for fluorescence and 0.0% for both comparators (Supplementary Table S6). For high-grade UC (n = 35), sensitivity was 71.4% (53.7–85.1), 39.4% (24.0–57.1) and 36.4% (21.8–54.0), respectively (Supplementary Table S7).

By T stage, fluorescence sensitivity was Ta 64.5%, Tis 100%, T1 75.0%, T2 85.7%, T4 0% and Tx 100%; in Ta tumors, DNA ploidy sensitivity was 16.1% and H&E sensitivity was 13.3% (4/30; H&E was not performed in one case), with comparators lower in most strata (Supplementary Table S8). Tis, T4 and Tx had 1–2 cases; these estimates are unstable.

3.4 Paired Comparative Analysis

Among 80 patients with all three assays (52 UC, 28 benign), fluorescence sensitivity was 73.1%, DNA ploidy 25.0% and H&E 23.1%; specificity was 78.6%, 100% and 100%; PPV was 86.4%, 100% and 100%; NPV was 61.1%, 41.8% and 41.2%; accuracy was 75.0%, 51.3% and 50.0%; Kappa was 0.485, 0.189 and 0.174 (Table 3). McNemar tests showed significant differences between fluorescence and DNA ploidy (χ^2=25.71, P < 0.001) and between fluorescence and H&E (χ^2=26.69, P < 0.001), but not between DNA ploidy and H&E (χ^2=0, P = 1.0).

Table 3
Paired Comparative Analysis of the Three Assays for Diagnostic Performance in UC.

Sensitivity	**Fluorescence staining**	**DNA ploidy analysis**	**H&E staining**
	73.1%	**25.0%**	**23.1%**
Specificity	78.6%	100%	100%
PPV	86.4%	100%	100%
NPV	61.1%	41.8%	41.2%
Accuracy	75.0%	51.3%	50.0%
Kappa	0.485	0.189	0.174

3.5 Excluded samples

Of 131 samples, 26 (19.8%) were excluded by QC. In a strict worst-case analysis, if all 26 excluded samples were UC and all were assumed negative on fluorescence, sensitivity would fall from 70.9% (39/55) to 48.1% (39/81). If all 26 were non-malignant and all were assumed positive on fluorescence, specificity would fall from 76.9% (30/39) to 46.2% (30/65). Only 6 excluded samples had fluorescence results (23.1%), all positive and without pathological confirmation; this strict worst-case is therefore likely overly pessimistic, but residual selection bias cannot be excluded.

4 Discussion

4.1 Main findings and positioning

This single-center prospective study used histopathology as the gold standard to evaluate the fluorescence assay as an auxiliary test for UC in 94 hematuria patients, with head-to-head comparison against DNA ploidy and H&E. The cohort had 55 UC (58.5%) and 39 benign lesions (41.5%), a population with high prior probability. Fluorescence was completed in all 94, with sensitivity 70.9%, specificity 76.9%, PPV 81.3%, NPV 65.2% and Kappa 0.466. DNA ploidy (n = 81) had sensitivity 24.5%, specificity 100.0%, PPV 100.0%, NPV 41.2% and Kappa 0.184; H&E (n = 80) had sensitivity 23.1%, specificity 100.0%,

PPV 100.0%, NPV 41.2% and Kappa 0.174. Fluorescence detected more UC but had lower specificity and an NPV of only 65.2%, insufficient to exclude malignancy alone.

The clinical positioning is therefore “auxiliary diagnosis,” not “replacement diagnosis” or “independent screening.” Fluorescence is rapid, non-invasive, requires only voided urine, produces results in approximately 20 minutes and can be performed on a conventional fluorescence microscope. However, specificity of 76.9% left 9 false positives, and NPV of 65.2% meant 16 of 46 fluorescence-negative patients had UC. For high-risk patients, negative fluorescence should not delay or cancel cystoscopy and upper tract evaluation. Reasonable uses include prioritizing cystoscopy when resources are limited, supplementing cytology for low-grade and Ta tumors, and providing low-resource-dependent information when rapid triage is needed or endoscopic acceptance is low. Because UC prevalence was 58.5%, PPV and NPV are prevalence-dependent and cannot be directly extrapolated to low-prevalence screening; the safety of negative results requires larger prospective validation.

4.2 Low-grade UC, Ta tumors and biological interpretation

Fluorescence showed a relative advantage in low-grade UC and Ta tumors. In 20 low-grade UC, sensitivity was 70.0% versus 0.0% for both comparators; in 31 Ta tumors, it was 64.5% versus 16.1% and 13.3%. This is consistent with the low sensitivity of conventional cytology in low-grade UC and suggests that morphology-only or DNA-content-only strategies may miss low-grade, low-stage tumors.

The reagent contains SYTOX Green and PI. SYTOX Green binds double-stranded DNA and produces green nuclear fluorescence; PI binds double-stranded DNA and RNA and produces orange-red fluorescence. Because voided exfoliated cells are mostly non-viable, both dyes label intact nucleic acid; green nuclear signal mainly reflects DNA, and orange-red cytoplasmic signal mainly reflects RNA. Tumor cells often have increased metabolic activity and RNA synthesis before obvious morphological changes, and PI-labeled cytoplasmic RNA may reflect this; SYTOX Green provides DNA and nuclear morphology information. Thus, the readout integrates nuclear DNA abnormality, increased cytoplasmic RNA and morphology. Compared with H&E (mainly morphology) and DNA ploidy (mainly DNA content), fluorescence may capture functional or metabolic changes earlier in low-grade tumors. This is consistent with previous acridine orange studies, but the RNA mechanism was not validated by quantitative RNA measurement or RNase A digestion; it remains a reasonable explanation, not mechanistic proof.

The “0.0%” sensitivity of DNA ploidy and H&E in low-grade UC should be interpreted cautiously. It cannot be concluded that these methods are completely ineffective; no positive cases were observed, suggesting potentially very low sensitivity with limited precision. Similarly, Ta sensitivity of 16.1% and 13.3% was clearly lower than fluorescence but with wide CIs. Low-grade and Ta cells have mild atypia and limited nuclear-to-cytoplasmic ratio increase, overlapping with normal or reactive urothelial cells; DNA ploidy may also be limited by shedding, preservation and near-diploid tumors. If reproduced, the relative advantage in low-grade and Ta tumors would be clinically valuable because this population is most easily missed by conventional cytology and most in need of non-invasive monitoring.

4.3 High-grade UC, T-stage subgroups and heterogeneity

In high-grade UC, fluorescence sensitivity was 71.4%, DNA ploidy 39.4% and H&E 36.4%. Fluorescence remained superior to both comparators in high-grade UC, and its sensitivity was similar to, and numerically slightly higher than, that in low-grade UC (71.4% vs 70.0%). Possible reasons for the absence of a substantial increase include the small high-grade sample (n = 35), more necrosis/hemorrhage/inflammation affecting smear quality, heterogeneity in RNA content and metabolic status, and subjectivity in combined morphological/fluorescence criteria. Larger samples and quantitative image analysis are needed.

T-stage subgroup analysis showed unstable estimates. Ta (n = 31) fluorescence sensitivity was 64.5%; Tis (n = 2) 100%; T1 (n = 12) 75.0%; T2 (n = 7) 85.7%; T4 (n = 1) 0%; Tx (n = 2) 100%. These are exploratory; T4 0% with one case cannot be interpreted as low T4 sensitivity, and Tis/Tx CIs are very wide. Upper tract UC was also a small subgroup (renal pelvis 2, ureter 2, combined 2); efficacy for upper tract UC cannot be evaluated. Future studies must expand upper tract cases and standardize specimen collection.

4.4 Paired comparison, missing assays and QC

The paired analysis in 80 patients (52 UC, 28 benign) partly controlled for tumor burden, specimen quality and patient composition. Fluorescence sensitivity was 73.1% versus 25.0% and 23.1%; McNemar tests were significant for fluorescence versus both comparators but not between comparators. However, DNA ploidy and H&E were not completed in all 94; some patients had prior CT urography and declined additional tests, so missingness was not completely random and may relate to preference, risk assessment, prior results and economic factors.

QC excluded 26/131 (19.8%). QC was performed before interpretation by personnel blinded to pathology, reducing interpretation bias, but exclusion reasons may be tumor-related. Worst-case analysis showed sensitivity could fall to 48.1% if all excluded samples were UC. Only 6 excluded samples had positive fluorescence without pathological confirmation, so major upward bias is unlikely, but residual selection bias cannot be excluded.

4.5 Strengths, limitations and clinical implications

Strengths include prospective design, histopathology gold standard, STARD 2015, fluorescence completed in all 94, synchronous comparators, blinding, randomized reading, third-pathologist arbitration, pre-defined QC, pre-study sample size estimation, 95% CIs and Kappa, paired analysis and worst-case analysis.

Limitations include single-center small sample (n = 94); small subgroups (upper tract UC, T2, Tis, T4, Tx); non-random missing comparators; 19.8% QC exclusion with worst-case sensitivity 48.1%; no healthy controls; few upper tract cases; unvalidated RNA mechanism; no cost-effectiveness, acceptance or outcome study; subjective interpretation criteria; and no assessment of whether results change cystoscopy scheduling, treatment or outcomes.

Clinically, the assay may be a more sensitive auxiliary test than DNA ploidy and H&E, especially for low-grade and Ta tumors. It requires only voided urine, takes approximately 20 minutes and uses a conventional fluorescence microscope, with lower resource dependence than cystoscopy. It may help prioritize cystoscopy, supplement cytology or support referral where resources are limited. However, NPV is 65.2% and specificity 76.9%; negative results cannot exclude UC and positive results cannot confirm it. High-risk patients should complete cystoscopy and upper tract evaluation regardless of fluorescence results.

Future research should include multicenter large-sample validation expanding upper tract UC, ≥T2, Tis and low-grade cases; paired or randomized comparisons of fluorescence plus cytology versus cytology alone; standardized scoring and AI-assisted reading; validation of PI cytoplasmic RNA by RNase A treatment or quantitative RNA; combination with FISH and other urinary markers; health-economic and acceptance studies; monitoring studies in non-muscle-invasive bladder cancer; and outcome studies.

5 Conclusion

In this single-center prospective study using histopathology as the gold standard, the tricyclic aromatic cation fluorescence urinary exfoliated-cell assay was evaluated as an auxiliary test for UC in 94 hematuria patients. Fluorescence was interpretable in all 94, with sensitivity 70.9% (57.9–81.2), specificity 76.9% (61.6–87.4), PPV 81.3%, NPV 65.2% and Kappa 0.466. Sensitivity was significantly higher than DNA ploidy (24.5%) and H&E (23.1%) (both $P < 0.001$); the comparators had 100% specificity in definitive-result samples but low sensitivity and limited NPV, with no difference between them ($P = 1.0$). Fluorescence showed relative advantages in low-grade UC (70.0% vs 0.0%/0.0%) and Ta tumors (64.5% vs 16.1%/13.3%), and was superior in high-grade UC (71.4% vs 39.4%/36.4%); Tis, T4, Tx and upper tract subgroups were small and exploratory.

The assay is an auxiliary and supplementary tool, not an independent screening tool, and cannot replace cystoscopy or pathology. NPV of 65.2% and specificity of 76.9% mean negative fluorescence cannot exclude malignancy and positive results cannot confirm diagnosis; high-risk patients should complete cystoscopy and upper tract evaluation regardless of fluorescence. The assay may provide supplementary information when cystoscopy resources are limited, acceptance is low or triage is needed, and may help address under-detection of low-grade and Ta tumors. However, this study is single-center with limited sample size, 26/131 (19.8%) QC exclusions, worst-case sensitivity 48.1%, non-random missing comparators, few upper tract cases, unvalidated RNA mechanism and no outcome assessment; extrapolation should be cautious. Future multicenter validation should expand upper tract UC, Tis, ≥T2 and low-grade cases, standardize interpretation, validate RNA mechanisms, explore combination strategies and conduct health-economic and outcome studies.

Abbreviations

Abbreviation

Definition
UC
Urothelial Carcinoma
SEN
Sensitivity
SPE
Specificity
PPV
Positive Predictive Value
H&E staining
Hematoxylin and Eosin staining

Declarations

Ethics approval and consent to participate

This single-center, prospective diagnostic study was approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine for Human Clinical and Research Projects (Approval No.: 2026□□□□404□). All enrolled patients provided written informed consent prior to participation. The study was conducted in accordance with the Declaration of Helsinki and relevant ethical guidelines for clinical research.

Consent for publication

This manuscript does not contain any individual person's data in identifiable form (including names, images, videos, or detailed case descriptions). All data are presented in anonymized form and cannot be traced back to individual patients. Therefore, this section is not applicable.

Competing interests

The authors declare that they have no competing interests. This study received no financial support, consulting fees, or any form of sponsorship from commercial entities, medical device manufacturers, or diagnostic reagent producers. All detection reagents used were routine clinical consumables, and no conflicts of interest exist with any manufacturer.

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Author Contribution

Study conceptualization and protocol design, H.X., Y.G., D.X. and H.H.; Data acquisition and patient enrollment management, H.X.; Fluorescence staining interpretation and pathological evaluation, Y.L., L.Z., X.L., S.L., K.D. and L.Y.; Statistical analysis and data interpretation, H.X.; Drafting and revision of the manuscript, H.X. and H.H. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request. Due to patient privacy and confidentiality concerns, the raw data have not been publicly archived in a public database. De-identified datasets used for statistical analysis may be made available to qualified researchers upon reasonable request to the corresponding author.

References

1. Rai BP, Luis Dominguez Escrig J, Vale L, et al. Systematic review of the incidence of and risk factors for urothelial cancers and renal cell carcinoma among patients with haematuria. Eur Urol. 2022;82(2):182–92.
2. Barocas DA, Lotan Y, Matulewicz RS, et al. Updates to microhematuria: AUA/SUFU guideline (2025). J Urol. 2025;213(5):547–57.
3. Wan X, Wang D, Zhang X, et al. Unleashing the power of urine-based biomarkers in diagnosis, prognosis and monitoring of bladder cancer (Review). Int J Oncol. 2025;66(3):18.
4. Kumar N, Talwar R, Nandy PR. Efficacy of voided urinary cytology and ultrasonography compared to cystoscopy in the detection of urinary bladder cancer. Afr J Urol. 2017;23(3):192–6.
5. Roth BL, Poot M, Yue ST, et al. Bacterial viability and antibiotic susceptibility testing with SYTOX green nucleic acid stain. Appl Environ Microbiol. 1997;63(6):2421–31.

6. Thakur S, Cattoni DI, Nöllmann M. The fluorescence properties and binding mechanism of SYTOX green, a bright, low photo-damage DNA intercalating agent. Eur Biophys J. 2015;44(5):337–48.
7. Rieger AM, Hall BE, Luong LT, et al. Conventional apoptosis assays using propidium iodide generate a significant number of false positives that prevent accurate assessment of cell death. J Immunol Methods. 2010;358(1):81–92.
8. Kantarjian HM, Barlogie B, Pershouse M, et al. Preferential expression of double-stranded ribonucleic acid in tumor versus normal cells: biological and clinical implications. Blood. 1985;66(1):39–46.
9. Martinez-Piñeiro JA, Muntanola P, Martin MG, et al. Fluorescence urinary cytology in bladder cancer. Eur Urol. 1977;3(3):142–4.
10. Luo Y, She DL, Xiong H, et al. Diagnostic value of liquid-based cytology in urothelial carcinoma diagnosis: a systematic review and meta-analysis. PLoS ONE. 2015;10(8):e0134940.
11. Liu R, Tian Z, Wang J, et al. Evaluation of acridine orange florescence in exfoliative urinary cytology for diagnosing bladder carcinoma. Int Urol Nephrol. 2012;44(5):1375–82.
12. de Oliveira MC, Caires HR, Oliveira MJ, et al. Urinary biomarkers in bladder cancer: where do we stand and potential role of extracellular vesicles. Cancers (Basel). 2020;12(6):1400.
13. Zhang Z, Wu ZL, Shen C et al. Exploring the influencing factors in urine cytology detection rates in urothelial carcinoma: an interpretable machine learning approach. J Clin Urol. 2024:1046–9. (in Chinese).
14. Tian ZT, Xu Y, Wang J, et al. Acridine orange fluorescence in diagnosis of bladder cancer. Chin J Urol. 2009;30(4):245–7. (in Chinese).

Figures

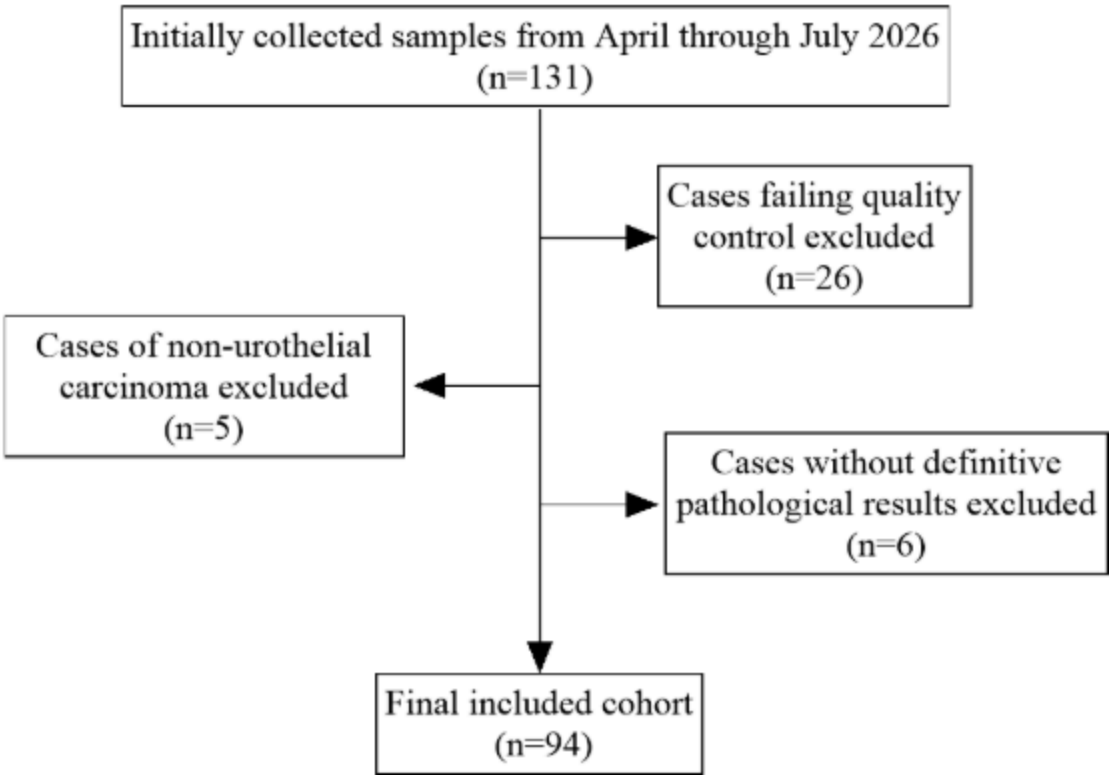


Figure 1

Flowchart of sample selection.

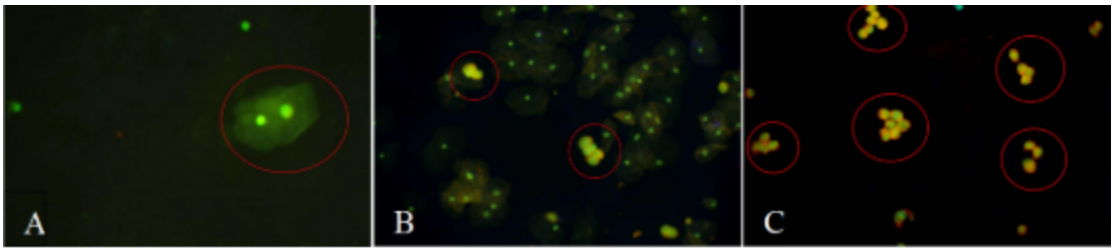


Figure 2

Representative fluorescence images of urinary exfoliated cells .

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarylistv10.30.docx