

Supplemental Table 1. Inclusion and exclusion criteria for clinical cohorts analyzed in this investigation.

Cohort	Inclusion Criteria	Exclusion Criteria
<i>Tumor Positive</i>	<ul style="list-style-type: none"> • Consented. • Pathologically confirmed UC present in urinary tract at time of collection. • Sample collected prior to surgical resection of lesion. 	<ul style="list-style-type: none"> • Prior history of pelvic radiation.
<i>Surveillance Negative</i>	<ul style="list-style-type: none"> • Consented. • UC patient with prior definitive surgical treatment and negative cystoscopy within 1 month of collection, and a second negative cystoscopy 3-12 months following the collection. 	<ul style="list-style-type: none"> • Prior history of pelvic radiation.
<i>Urology Control</i>	<ul style="list-style-type: none"> • Consented. 	<ul style="list-style-type: none"> • Pathologic, cystoscopic, or imaging evidence of, or a known history of UC. • Pathologic, imaging, or laboratory evidence of current or prior prostate or renal cancer • Hematuria not evaluated to standard of care.

Supplemental Table 2. AUA Hematuria risk stratification of hematuria subjects with available data ($n = 129$) among train and validation cohorts.

AUA Hematuria Risk Group	uCGP Cohorts	Reference from 15,000+ patient meta-analysis [3]
High	85.3%	83.6%
Intermediate	13.2%	11.8%
Low	1.6%	4.6%

Supplemental Table 3. Test sensitivity and specificity performance stratified by institution.
 *Sample size numbers listed in order of train, validation (aggregated performance across sites),
 CURE (validation), MGH (validation), and OSU (validation) cohorts.

	Train	Validation	CURE	MGH	OSU
% Sensitivity					
Initial Diagnosis					
Overall (56/22/10/6/6)*	98 (90-100)	96 (77-100)	90 (56-100)	100 (54-100)	100 (54-100)
High Grade (42/14/6/3/5)	100 (92-100)	100 (77-100)	100 (54-100)	100 (29-100)	100 (48-100)
Low Grade (14/8/4/3/1)	93 (66-100)	88 (47-100)	75 (19-99)	100 (29-100)	100 (3-100)
NMIBC (38/18/8/6/4)	97 (86-100)	94 (73-100)	88 (47-100)	100 (54-100)	100 (40-100)
MIBC (16/4/2/-/2)	100 (79-100)	100 (40-100)	100 (16-100)	-	100 (16-100)
Surveillance					
Overall (38/48/16/24/8)	74 (57-87)	65 (50-78)	69 (41-89)	58 (37-78)	75 (35-97)
High Grade (17/17/4/7/6)	88 (64-99)	77 (50-93)	75 (19-99)	71 (29-96)	83 (36-100)
Low Grade (18/31/12/17/2)	56 (31-79)	58 (39-76)	67 (35-90)	53 (28-77)	50 (1-99)
NMIBC (27/47/16/24/7)	67 (46-84)	64 (49-77)	69 (41-89)	58 (37-78)	71 (29-96)
MIBC (4/1/-/-/1)	100 (40-100)	100 (3-100)	-	-	100 (3-100)
Upper Tract UC					
Overall (7/2/2/-/-)	100 (40-100)	100 (16-100)	100 (16-100)	-	-
% Specificity					
Initial Diagnosis					
All urology controls (139/96/48/47/1)*	96 (91-98)	90 (82-95)	90 (77-97)	89 (77-97)	100 (3-100)
Controls with UTI (23/10/9/1/-)	100 (85-100)	90 (56-100)	89 (52-100)	100 (3-100)	-
Controls with hematuria (70/37/32/5/-)	96 (88-99)	87 (71-96)	88 (71-97)	80 (28-100)	-
Controls with BPH (22/26/19/7/-)	96 (77-100)	85 (65-96)	90 (67-99)	71 (29-96)	-
Controls with Leukocytes (48/14/14/-/-)	98 (89-100)	86 (57-98)	86 (57-98)	-	-
Controls with LUTS (29/20/13/7/-)	93 (77-99)	80 (56-94)	85 (55-98)	71 (29-96)	-
Surveillance					
Hx UC with negative cystoscopy (100/82/35/44/3)	89 (81-94)	89 (80-95)	91 (77-98)	86 (73-95)	100 (29-100)
% Pos Predictive Value					
Hematuria/Initial diagnosis (10% Prevalence)	72	51	49	51	100
Recurrence monitoring (20% Prevalence)	63	59	67	52	100
% Neg Predictive Value					
Hematuria/Initial diagnosis (10% Prevalence)	100	99	99	100	100
Recurrence monitoring (20% Prevalence)	93	91	92	89	94

Supplemental Table 4. Comparison of pre- and post-test probability of UC given diagnostic likelihood ratios (DLR) for positive and negative tests. Values taken from Woldu *et. al.* 2021 [25]. CI denotes 95% confidence intervals.

Pre-test prob. of UC	Positive likelihood ratio	Post-test prob. of UC	Negative likelihood ratio	Post-test prob. of UC
NMP22				
0.5%		1.5		0.2
1.0%	3.05	3.0	0.4	0.4
3.0%	CI: (2.28-4.10)	8.6	CI: (0.32-0.50)	1.2
10.0%		25.3		4.3
BTA				
0.5%		1.3		0.2
1.0%	2.52	2.5	0.47	0.5
3.0%	CI: (1.86-3.41)	7.2	CI: (0.37-0.61)	1.4
10.0%		21.9		5.0
UroVysion (FISH)				
0.5%		2.5		0.2
1.0%	5.02	4.8	0.42	0.4
3.0%	CI: (2.93-8.60)	13.4	CI: (0.30-0.59)	1.3
10.0%		35.8		4.5
ImmunoCyt				
0.5%		1.7		0.1
1.0%	3.49	3.4	0.29	0.3
3.0%	CI: (2.82-4.32)	9.7	CI: (0.20-0.41)	0.9
10.0%		27.9		3.1
CxBladder				
0.5%		1.1		0.1
1.0%	2.14	2.1	0.21	0.2
3.0%		6.2		0.6
10.0%		19.2		2.3
AssureMDx				
0.5%		3.2		0.0
1.0%	6.6	6.3	0.07	0.1
3.0%		17.0		0.2
10.0%		42.3		0.8
GeneXpert				
0.5%		2.4		0.1
1.0%	4.98	4.8	0.27	0.3
3.0%		13.3		0.8
10.0%		35.6		2.9
Cytology				
0.5%		3.7		0.2
1.0%	7.67	7.2	0.35	0.4
3.0%	CI: (4.51-13.03)	19.2	CI: (0.18-0.68)	1.1
10.0%		46.0		3.7
UroAmp				
0.5%		4.4		0.03
1.0%	9.2	8.5	0.05	0.05
3.0%	CI: (5.1-16.6)	22.1	CI: (0.01-0.34)	0.16
10.0%		50.5		0.56

Supplemental Table 5. UroAmp test features predictive of tumor grade and stage. Results calculated from tumor-positive subjects in aggregated train and validation cohorts ($n = 164$). Abbreviations: Odds ratio (OR), 95% confidence interval (CI), High grade (HG), Low grade (LG), Muscle-invasive bladder cancer (MIBC), Non-muscle-invasive bladder cancer (NMIBC), Carcinoma *in situ* (CIS), Loss of heterozygosity (LOH). (*) Mutation only observed in one condition. (^{WT}) Unmutated gene. (p-adj) denotes false discovery rate adjusted P -value.

Comparison	Feature	OR	CI	P -value	P -adj	Positives
<i>HG vs. LG</i>	* <i>CDKN1A</i>	> 30	–	0.0348	0.0547	6
	<i>TERT, TP53, FGFR3^{WT}</i>	28	(3.8, 220)	< 0.0001	< 0.0001	27
	Gene CNV	14	(4.7, 42)	< 0.0001	< 0.0001	45
	<i>RB1</i>	13	(1.7, 100)	0.0019	0.0052	15
	<i>TP53</i>	9.9	(3.9, 25)	< 0.0001	< 0.0001	49
	Copy-neutral LOH	6.6	(2.6, 17)	< 0.0001	0.0001	40
	Whole-Genome Aneuploidy	4.9	(2.2, 11)	< 0.0001	0.0001	50
	<i>TERT, FGFR3^{WT}</i>	3.4	(1.7, 6.9)	0.0005	0.0019	58
	INDELS	2.8	(1.4, 5.5)	0.0046	0.0100	56
	<i>TERT</i>	2.0	(1.1, 3.9)	0.0347	0.0547	99
	<i>FGFR3</i>	0.33	(0.17, 0.65)	0.0011	0.0035	61
<i>FGFR3, TERT^{WT}</i>	0.22	(0.08, 0.64)	0.0037	0.0090	20	
<i>MIBC vs. NMIBC</i>	<i>TP53</i>	7.4	(2.9, 19)	< 0.0001	0.0004	46
	<i>TERT, TP53, FGFR3^{WT}</i>	6.5	(2.5, 17)	0.0002	0.0028	25
	Gene CNV	4.9	(2.0, 12)	0.0008	0.0063	41
	Whole-Genome Aneuploidy	4.4	(1.8, 11)	0.0015	0.0093	48
	<i>TERT, FGFR3^{WT}</i>	3.6	(1.5, 8.8)	0.0050	0.0242	53
	<i>TERT</i>	3.0	(1.1, 8.6)	0.0429	0.1358	94
	Copy-neutral LOH	2.4	(0.98, 6.0)	0.0731	0.1949	38
	<i>FGFR3</i>	0.33	(0.12, 0.93)	0.0429	0.1358	61
	* <i>FGFR3, TERT^{WT}</i>	0.00	–	0.0453	0.1358	20
<i>HG T1+ vs. HG Ta/CIS</i>	<i>TP53</i>	6.0	(2.3, 16)	0.0002	0.0025	40
	<i>TERT, TP53, FGFR3^{WT}</i>	3.2	(1.1, 9.2)	0.0307	0.1880	24
<i>CIS vs. Ta</i>	<i>TERT, TP53, FGFR3^{WT}</i>	9.9	(1.8, 53)	0.0176	0.2381	7
	<i>KMT2A</i>	6.4	(1.3, 31)	0.0380	0.2568	9
	<i>TERT, FGFR3^{WT}</i>	5.4	(1.4, 21)	0.0164	0.2381	27
	<i>TP53</i>	5.2	(1.3, 21)	0.0334	0.2568	15
	<i>FGFR3</i>	0.26	(0.05, 1.3)	0.1027	0.3082	49

Supplemental Table 6. Clinical demographics of the long-term outcomes cohort used for uCGP predicted recurrence risk ($n = 55$).

	Recurrence Status	
	No	Yes
Primary Grade		
HG	23	5
LG	22	5
CIS	1	0
T1	8	1
T2	1	0
T3	1	0
Ta	33	9
unavailable	1	0
AUA Recurrent Risk		
high	13	4
intermediate	16	2
low	13	4
unavailable	3	0
Prior Intravesical Therapy		
no	24	6
yes	21	4
Age		
<70	20	3
≥70	25	7
Sex		
F	9	2
M	36	8
Smoking History		
current	7	0
former	22	7
never	16	3
Race		
Asian	5	0
Black or African American	0	1
Hispanic or Latino	1	1
White	38	8
other	1	0

Supplemental Table 7. Univariable Cox proportional-hazard regression analysis of UroAmp recurrence risk groups and clinical risk factors. For stage, T1+ indicated the grouping of patients with T1, T2, and T3 disease.

Feature	Univariable	
	HR (95% CI)	P-value
UroAmp Recurrence Risk	6.2 [1.6 - 24]	0.008
≥70 vs. <70	2.1 [0.55 - 8.3]	0.272
Smoking: Ever vs. Never	0.74 [0.19 - 2.9]	0.658
Male vs. Female	0.90 [0.19 - 4.3]	0.897
Primary Grade: HG vs. LG	1.0 [0.29 - 3.5]	0.995
Primary Stage: T1+ vs. Ta	0.28 [0.04 - 2.2]	0.228

Supplemental Table 8. Comparison of urine cytology and UroAmp in $n = 66$ urine specimens (18 initial diagnosis, 48 surveillance). Urine cytology returned atypical results in 21 of these 66 patients. Values in parenthesis denote 95% confidence intervals. Positive and negative predictive values were computed with an assumption of 15% prevalence.

	UroAmp	Urine Cytology (atypical withheld)	Urine Cytology (atypical as negative)	Urine Cytology (atypical as positive)	UroAmp (atypical cytology)
% Sensitivity (95% CI)					
Overall ($n = 19$)	79 (54 - 94)	25 (6 - 57)	16 (3 - 40)	53 (29 - 76)	86 (42 - 100)
High Grade ($n = 7$)	100 (59 - 100)	50 (7 - 93)	29 (4 - 71)	71 (29 - 96)	100 (29 - 100)
Low Grade ($n = 12$)	67 (35 - 90)	13 (0.3 - 53)	8 (0.2 - 39)	42 (15 - 72)	75 (19 - 99)
% Specificity (95% CI)					
Overall ($n = 47$)	83 (69 - 92)	94 (80 - 99)	96 (86 - 100)	66 (51 - 79)	71 (42 - 92)
% PPV	45	42	39	21	35
% NPV	96	88	87	89	97