DNA Methylation in Urine and Feces Indicative of Eight Major Human Cancer Types Globally

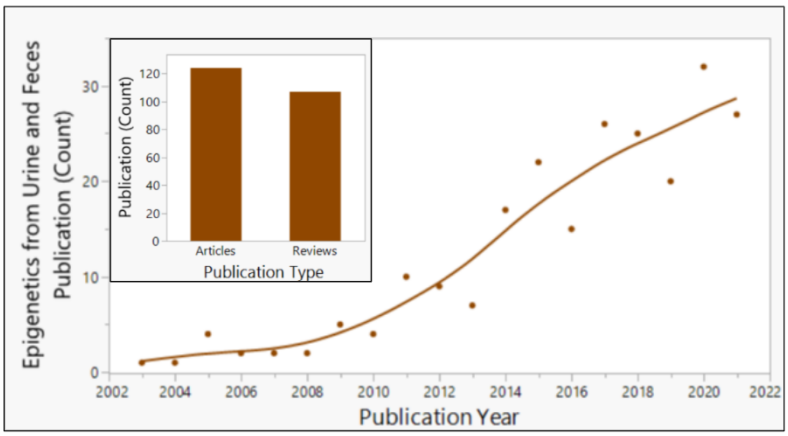
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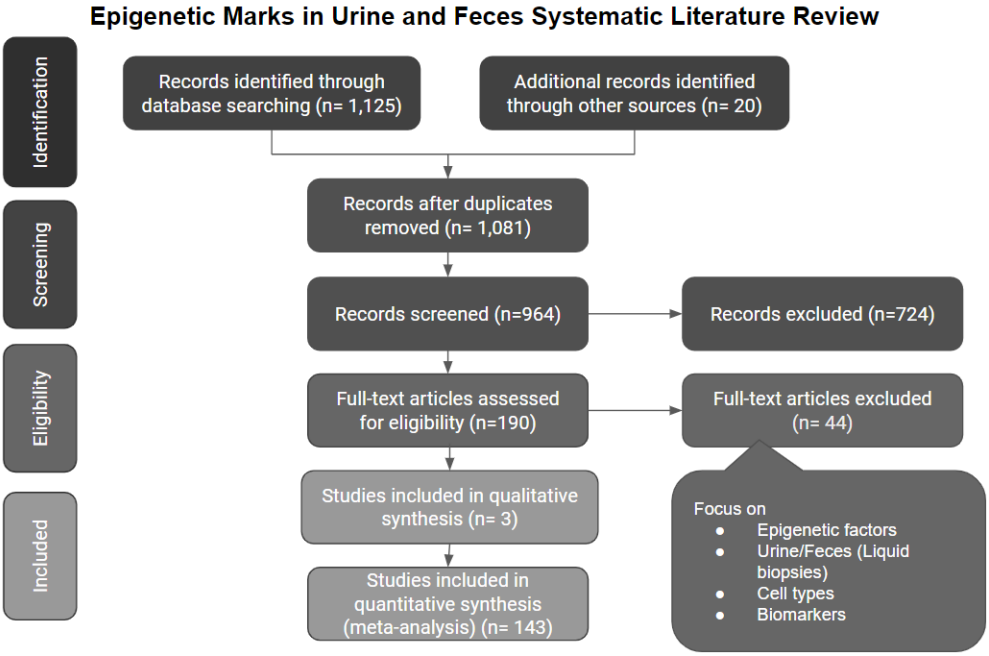
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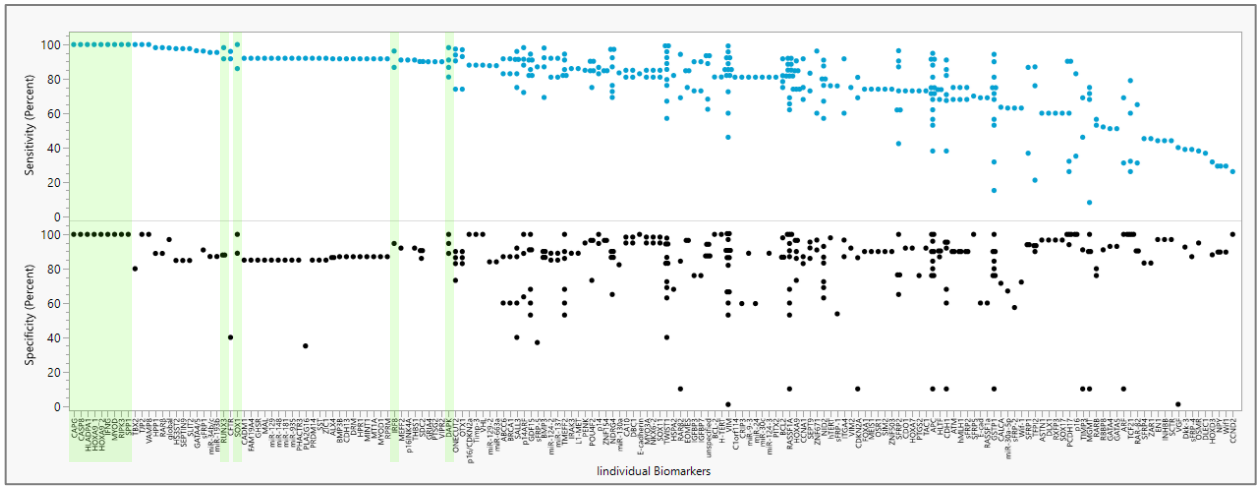
Supplemental Material



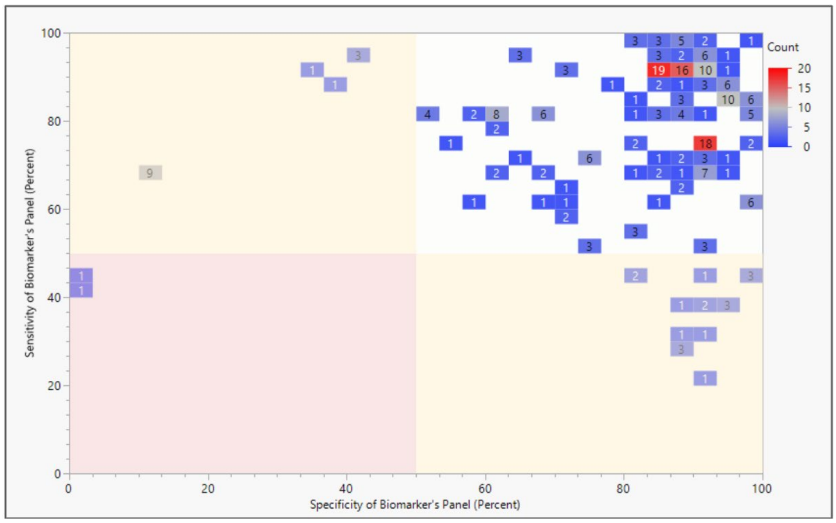
Supplemental Figure S1. Publication Trends in Epigenetics in Urine and Feces PRISMA Literature Review Search. (a) Line graph showing publications (count) over time of epigenetic marks in urine and feces. (b) The number of articles and reviews are compared in a histogram.



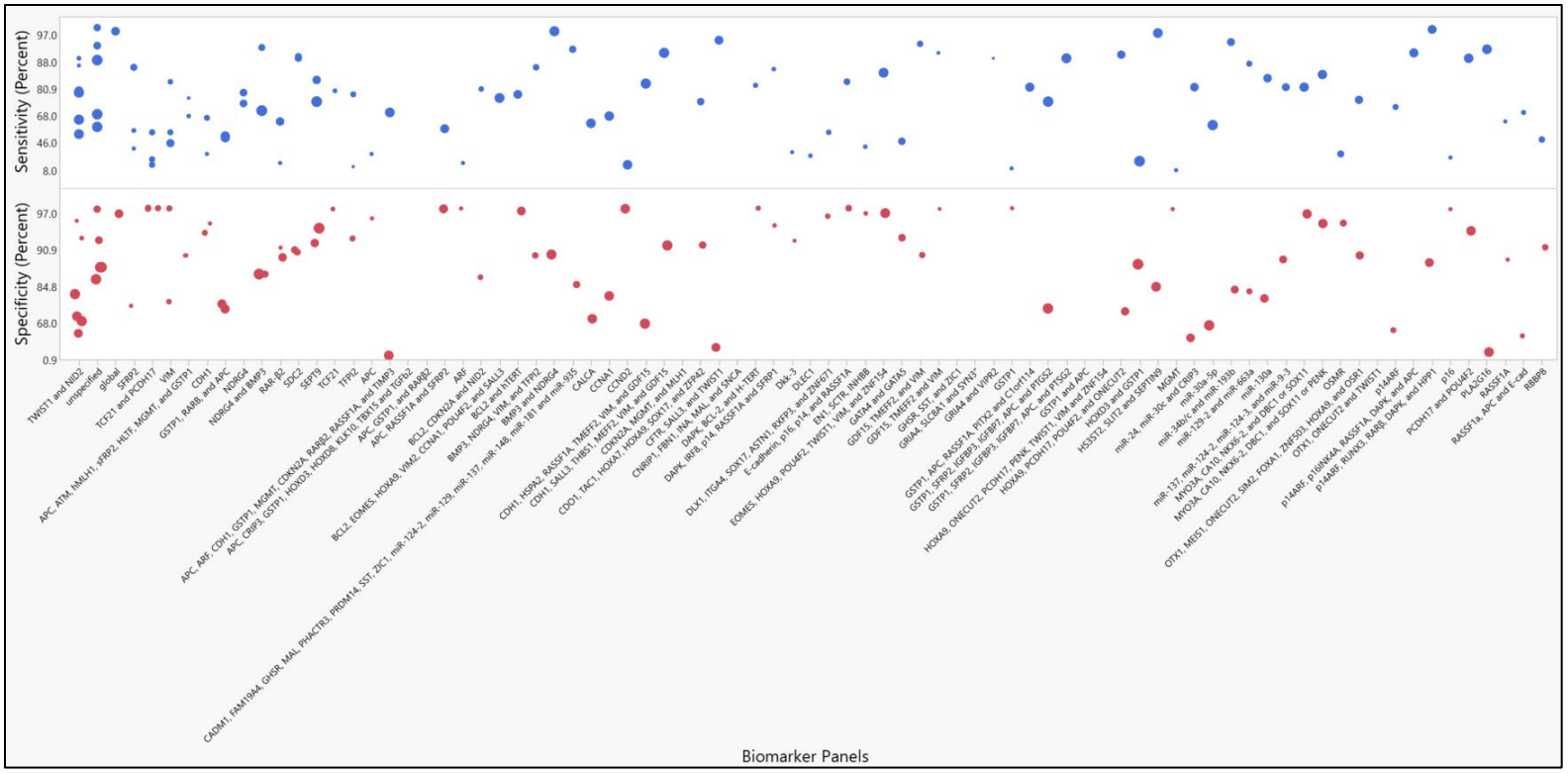
Supplemental Figure S2. PRISMA Systematic Literature Review Diagram of Liquid Biopsy (Urine and Feces) Epigenetic Marks. Key words: epigen* AND (fecal OR feces OR urine) AND cell AND biomarker



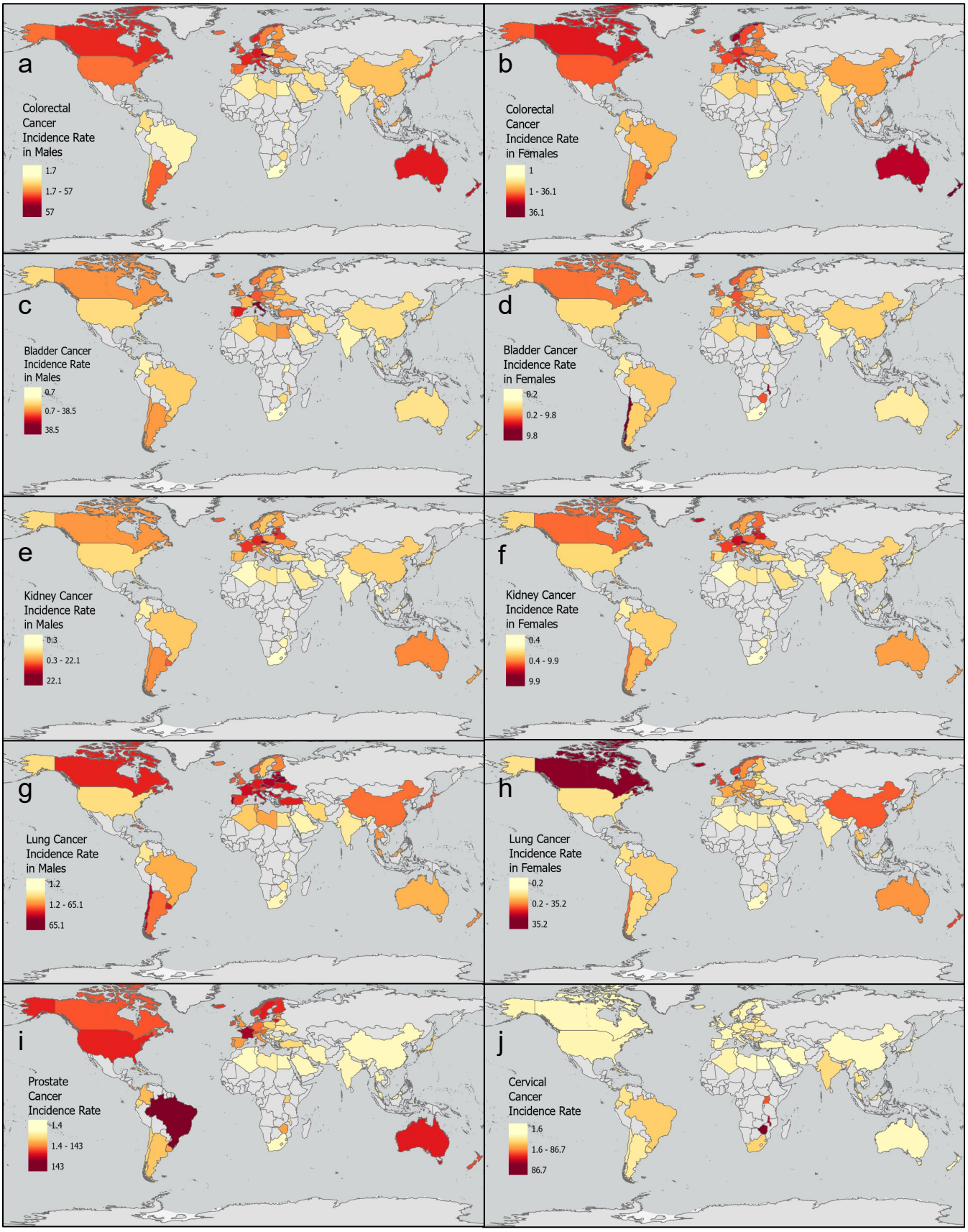
Supplemental Figure S3. Rank-ordered Epigenetic Biomarker Sensitivity Urine and Feces by Biomarker and Disease. Percent sensitivity and specificity reported by screening each epigenetic biomarker panel in liquid biopsy (urine and feces) are disaggregated into each biomarker**. Green highlight bars represent biomarker panels and individual biomarkers with the highest sensitivity and specificity given cancer type or replicability.
**Each biomarker represents the percent sensitivity and specificity reported from each panel in which it was present.



Supplemental Figure S4. Epigenetic Biomarker Sensitivity and Specificity in Liquid Biopsy (Urine and Feces) by Publication Count. Percent sensitivity and specificity are plotted against each other in a heatmap disaggregated into individual biomarkers* from the reported studies of liquid biopsy (urine and feces) represented in panel a. The red highlighted section represents sensitivity + specificity below the preferred 1.5 threshold, the yellow sections highlight studies meeting the 1.5 threshold, and the green section shows the studies above the 1.5 threshold.
*Each biomarker represents the percent sensitivity and specificity reported from each panel in which it was present.



Supplemental Figure S5. Epigenetic Biomarker Sensitivity and Specificity in Liquid Biopsy (Urine and Feces) scaled by Sample Size. Biomarker panels are plotted by percent sensitivity (blue) and specificity (red) and sample size (patient count) ranging from n = 4 to n = 408.


a
Colorectal Cancer Incidence Rate in Males
1.7
1.7 - 57
57
b
Colorectal Cancer Incidence Rate in Females
1
1 - 36.1
36.1
c
Bladder Cancer Incidence Rate in Males
0.7
0.7 - 38.5
38.5
d
Bladder Cancer Incidence Rate in Females
0.2
0.2 - 9.8
9.8
e
Kidney Cancer Incidence Rate in Males
0.3
0.3 - 22.1
22.1
f
Kidney Cancer Incidence Rate in Females
0.4
0.4 - 9.9
9.9
g
Lung Cancer Incidence Rate in Males
1.2
1.2 - 65.1
65.1
h
Lung Cancer Incidence Rate in Females
0.2
0.2 - 35.2
35.2
i
Prostate Cancer Incidence Rate
1.4
1.4 - 143
143
j
Cervical Cancer Incidence Rate
1.6
1.6 - 86.7
86.7

Supplemental Figure S6. Cancer Incidence Hotspots. Maps show incidence rates by country for colon and rectal combined to colorectal (a & b), bladder (c & d), kidney (e & f), lung (g & h), prostate (i), and cervical cancer (j). Incidence rates (per 100,000 people) reported by the Global Cancer Observatory are scaled by color, by sex, and country.

Supplemental Table S1. Metadata of Epigenetic Biomarker Panel Studies include in this Review

No.	Disease Details	Disease	Liquid biopsy specimen (i.e., Urine, Feces)	Sample size	Centrifuged sample	Fragment copies Details	RNA or DNA concentration (ug)	RNA or DNA concentration (uL)	Calculated RNA or DNA concentration (ug/mL)	RNA or DNA concentration Details	Extraction Method	Biomarker(s)	Epigenetic marker type	Epigenetic Assay	Epigenetic Frequency	Statistical Methods	Sensitivity (disease as positive) Details	Specificity (control as negative) Details	Patient (#) Details	Controls (#) Details	Geographic region(s)	Latitude (decimal)	Longitude (decimal)	Source	Comment
1	colorectal cancer, colorectal neoplasia	Colorectal Cancer	feces	36 g	12 mL	75 copies per reaction volume					direct gene capture from fecal supernatant	BMP3, NDRG4, vimentin, and TFPI2 plus mutant KRAS quantitative fecal hemoglobin	methylation	rapid bisulfite treatment process QuARTS method pre-commercial prototype sDNA assay		95% CI Fisher's exact binomial distribution logistic regression	Neoplasm: 85% Adenomas: 82% Cancer: 87% stage I and 2 both (86%), III (100%), and IV (75%) proximal: 92% distal: 81%	90% cutoff for positive	52	46	Rochester, Minnesota	44.016369	-92.475395	Ahlquist et al., 2012	
2	bladder cancer, urothelial carcinomas	Bladder Cancer	urine					1		1 ug Total RNA, 20 ng cDNA	ZR Urine DNA Isolation Kit ™	SFRP5	methylation	bisulfite modified EZ-DNA methylation kit 1 µg of total RNA Promega reverse transcription system Taq Polymerase ethidium bromide, UV light Reference gene: GAPDH		p-value <0.05 Fisher exact test	70%	0% control	20	5	Aachen, Germany	50.775555	6.083611	Antony et al., 2010	
3	urothelial bladder cancer	Bladder Cancer	urine				2			2 µL bisulfite-treated DNA / 50uL	ZR Urine DNA Isolation KitTM	CDH1 promoter	methylation	~200 ng DNA/20 µL Bisulfite conversion EZ DNA Methylation™ Kit MS-PCR Taq Polymerase	CDH1: 68.8% p14ARF: 72.9% Control CDH1: 5.7% Control p14ARF: 37.1%	p-value 0.05 normal data distribution: Kolmogorov–Smirnov test nonparametric Spearman's rank correlation and	CDH1: 67.4% urine cytology: 34.9%	CDH1: 93.9% urine cytology: 93.9%	48	35	Samsun, Turkey	41.292782	36.33128	Bayramov et al., 2018	

																Mann–Whitney U-test k test									
3	urothelial bladder cancer	Bladder Cancer	urine								ZR Urine DNA Isolation KitTM	p14ARF promoter	methylation	~200 ng DNA/20 µL Bisulfite conversion EZ DNA Methylation™ Kit MS-PCR Taq Polymerase	CDH1: 68.8% p14ARF: 72.9% Control CDH1: 5.7% Control p14ARF: 37.1%	p-value 0.05 normal data distribution: Kolmogorov–Smirnov test nonparametric Spearman's rank correlation and Mann–Whitney U-test k test	p14ARF: 72.1% urine cytology: 34.9%	p14ARF: 63.6% urine cytology: 93.9%	48	35	Samsun, Turkey	41.292782	36.33128	Bayramov et al., 2018	
4	colorectal cancer	Colorectal Cancer	feces	4 g		normal patients (median = 936 genome equivalents, range = 33–18 560 genome equivalents) and cancer patients (median = 1014 genome equivalents, range = 32–3700 genome equivalent					4 g stool sample, 20 µ L of RNase A Total DNA	Vimentin exon 1	methylation	vimentin fragments acrylamide gel–based affinity capture MS-PCR SybrGreen Mastermix		95% CI log-odds ratios Wald-type test p <0.05	46% stage I or II: 43% stage II or IV: 50% proximal to the splenic flexure: 46% distal to the splenic flexure: 45% ~15 methylated cell detection limit	90%	94	198	Cleveland, Ohio	41.505493	-81.68129	Chen et al., 2005	
4	colorectal cancer	Colorectal Cancer	feces								4 g stool sample, 20 µ L of RNase A Total DNA	Vimentin exon 1	methylation	vimentin fragments acrylamide gel–based affinity capture MS-PCR SybrGreen Mastermix		95% CI log-odds ratios Wald-type test p <0.05	46% stage I or II: 43% stage II or IV: 50% proximal to the splenic flexure: 46% distal to the splenic flexure:	90%	94	198	Burlington, Massachusetts	42.508045	-71.197929	Chen et al., 2005	

																	45% ~15 methylated cell detection limit							
5	bladder cancer	Bladder Cancer	urine	30-50 mL			0.5		0.0125	<500 ng of urinary cfDNA		global	methylation	500 ng of urinary cfDNA KAPA HTP Library Preparation Kit library preparation bisulfite sequencing Illumina HiSeq 2500 sequencer analysis pipeline Methy-Pipe	global methylation range: 61.1% - 73.5% prportional cell contributions: blood cells: 52% (0–84%) kidney: 32% (7–100%) urothelium: 5% (0–12%)				26 samples from 11 renal transplant patients 5 samples from 2 hematopoietic stem cell transplant (HSCT) patients		Hong Kong, China	22.302711	114.177216	Cheng et al., 2017	
6	bladder cancer	Bladder Cancer	urine	50 mL						100 uL DNA (outlier)	centrifuged, pellet washed twice with PBS buffer QIAamp DNA Mini Kit	MYO3A, CA10, NKX6-2, and DBC1 or SOX11	methylation	bisulfite conversion EpiTect Bisulfite Kits quantitative methylation-specific real-time PCR (qMSP) internal control: mC-LESS		receiver operating characteristics (ROC) curve of the assay calculating the area under the curve (AUC) of ROC $p < 0.05$	MYO3A, CA10, NKX6-2, and DBC1 or SOX11 panel: 81%	MYO3A, CA10, NKX6-2, and DBC1 or SOX11 panel: 97%	128	110	Houston, Texas	29.749907	-95.358421	Chung et al., 2011	All biomarkers: "MYO3A, CA10, SOX11, NKX6-2, PENK, and DBC1"
6	bladder cancer	Bladder Cancer	urine								centrifuged, pellet washed twice with PBS buffer QIAamp DNA Mini Kit	MYO3A, CA10, NKX6-2, and DBC1 or SOX11	methylation	bisulfite conversion EpiTect Bisulfite Kits quantitative methylation-specific real-time PCR (qMSP) internal control: mC-LESS		receiver operating characteristics (ROC) curve of the assay calculating the area under the curve (AUC) of ROC $p < 0.05$	MYO3A, CA10, NKX6-2, and DBC1 or SOX11 panel: 81%	MYO3A, CA10, NKX6-2, and DBC1 or SOX11 panel: 97%	128	110	Dallas, Texas	32.779167	-96.808891	Chung et al., 2011	All biomarkers: "MYO3A, CA10, SOX11, NKX6-2, PENK, and DBC1"
6	bladder cancer	Bladder Cancer	urine								centrifuged, pellet washed twice with PBS buffer QIAamp DNA Mini Kit	MYO3A, CA10, NKX6-2, DBC1, and SOX11 or PENK	methylation	bisulfite conversion EpiTect Bisulfite Kits quantitative methylation-specific real-time PCR (qMSP) internal control: mC-LESS		receiver operating characteristics (ROC) curve of the assay calculating the area under the curve (AUC) of	MYO3A, CA10, NKX6-2, DBC1, and SOX11 or PENK panel: 85%	MYO3A, CA10, NKX6-2, DBC1, and SOX11 or PENK panel: 95%	128	110	Houston, Texas	29.749907	-95.358421	Chung et al., 2011	All biomarkers: "MYO3A, CA10, SOX11, NKX6-2, PENK, and DBC1"

																ROC p < 0.05									
6	bladder cancer	Bladder Cancer	urine								centrifuged, pellet washed twice with PBS buffer QIAamp DNA Mini Kit	MYO3A, CA10, NKX6-2, DBC1, and SOX11 or PENK	methylation	bisulfite conversion EpiTect Bisulfite Kits quantitative methylation-specific real-time PCR (qMSP) internal control: mC-LESS		receiver operating characteristics (ROC) curve of the assay calculating the area under the curve (AUC) of ROC p < 0.05	MYO3A, CA10, NKX6-2, DBC1, and SOX11 or PENK panel: 85%	MYO3A, CA10, NKX6-2, DBC1, and SOX11 or PENK panel: 95%	128	110	Dallas, Texas	32.779167	-96.808891	Chung et al., 2011	All biomarkers: "MYO3A, CA10, SOX11, NKX6-2, PENK, and DBC1"
7	bladder cancer	Bladder Cancer	urine							1.5 mg of total RNA (outlier)	centrifuged, pellet washed twice with phosphate-buffered solution phenol–chloroform procedure	GDF15, TMEFF2, and VIM CpG island-containing genes	methylation	bisulfite treatment real-time quantitative MSP (qMSP) TaqMan Universal PCR Master Mix No AmpErase UNG Reference gene: ALU	GDF15: 24/51 TMEFF2: 32/51 VIM: 40/51	Mann-Whitney Test p < 0.001	94%	90%	51 BlCa patients	20 healthy individuals, 19 renal cancer patients 20 prostate cancer patients	Lisbon, Portugal	38.736946	-9.142685	Costa et al., 2010	
8	non-muscle invasive bladder cancer	Bladder Cancer	urine	10 mL							centrifuged, pellet analyzed	15 (proprietary)	methylation	methylation-sensitive restriction enzyme digestion real-time PCR Bladder EpiCheckTM software		sensitivity analysis discrimination area under the receiver operating curve 95% CI bias boostrapping multivariate analysis calibration plots robustness differential area under the curve	any cancer: 67.3% high-grade cancer: 88.9%	any cancer or high-grade cancer: 88% negative predictive value (NPV) for any cancer: 94.4% high-grade cancer NPV: 99.3% EpiScore TM positive threshold : ≥60	357		Vienna, Austria	48.210033	16.363449	D'Andrea et al., 2019	
9	bladder cancer	Bladder Cancer	urine	50 mL								transcription factor 21(TCF21)	methylation			p<0. 05			80	62 non-bladder cancer patients 40 healthy	Fujian Quanzhou, China	25.721292	119.384155	Ding et al., 2019	Article from ILL unreadable

																				individuals					
10	colorectal cancer	Colorectal Cancer	feces				0.05			50 ng/µl DNA	QIAamp DNA Stool Mini Kit	GRIA4, SLC8A1 and SYN3 CpG islands	methylation	bisulfite conversion digital PCR methylation percentage BD Accuri C6	>1% of methylation at least one of the three markers: 87.5% GRIA4 79.2% SLC8A1: 70.8% SYN3: 62.5%	logistic regression model calculate the area under the curve (AUC)			24		Cagliari, Sardinia, Italy	39.227779	9.111111	Fadda et al., 2018	
10	colorectal cancer	Colorectal Cancer	feces								QIAamp DNA Stool Mini Kit	GRIA4, SLC8A1 and SYN3 CpG islands	methylation	bisulfite conversion digital PCR methylation percentage BD Accuri C6	>1% of methylation at least one of the three markers: 87.5% GRIA4 79.2% SLC8A1: 70.8% SYN3: 62.5%	logistic regression model calculate the area under the curve (AUC)			24		Sassari, Italy	40.725925	8.555683	Fadda et al., 2018	
11	bladder cancer	Bladder Cancer	urine			at least 10 copies of the β-actin gene						TWIST1 and NID2	methylation	real-time methylation-specific polymerase chain reaction (MS-PCR) reference gene: β-actin		95% CI areas under the curve TWIST1: 0.67 NID2: 0.64 combined TWIST1 and NID2: 0.66	positive threshold: 67% smokers: 78%	positive threshold : 69%	209		Durham, North Carolina	35.994034	-78.898621	Fantony et al., 2015	
11	bladder cancer	Bladder Cancer	urine									TWIST1 and NID2	methylation	real-time methylation-specific polymerase chain reaction (MS-PCR) reference gene: β-actin		95% CI areas under the curve TWIST1: 0.67 NID2: 0.64 combined TWIST1 and NID2: 0.66	positive threshold: 67% smokers: 78%	positive threshold : 69%	209		Norfolk, Virginia	36.850769	-76.285873	Fantony et al., 2015	
12	bladder cancer	Bladder Cancer	urine			at least 10 copies of the β-actin gene						TWIST1 and NID2	methylation	real-time methylation-specific polymerase chain reaction (MSPCR) reference gene: β-actin	positive cytology: AUC 0.704 positive cytology and DNA methylatio	likelihood ratio Areas under the curve (AUC) Receiver operator characteri	positive cytology and DNA methylation assay: 0.57 current smokers:	positive cytology and DNA methylation assay: 0.72	172		Durham, North Carolina	35.994034	-78.898621	Fantony et al., 2017	

															n assay: AUC 0.773 p < 0.001 negative cytology: AUC 0.558 negative cytology and DNA methylation assay: AUC 0.697 p = 0.003	stic (ROC) curves	78% compared to former: 56% non-smokers: 44%								
12	bladder cancer	Bladder Cancer	urine									TWIST1 and NID2	methylation	real-time methylation-specific polymerase chain reaction (MSPCR) reference gene: β-actin	positive cytology: AUC 0.704 positive cytology and DNA methylation assay: AUC 0.773 p < 0.001 negative cytology: AUC 0.558 negative cytology and DNA methylation assay: AUC 0.697 p = 0.003	likelihood ratio Areas under the curve (AUC) Receiver operator characteristic (ROC) curves	positive cytology and DNA methylation assay: 0.57 current smokers: 78% compared to former: 56% non-smokers: 44%	positive cytology and DNA methylation assay: 0.72	172		Norfolk, Virginia	36.850769	-76.285873	Fantony et al., 2017	
13	bladder cancer	Bladder Cancer	urine				0.5			500 ng DNA	centrifuged, pellet washed with PBS buffer DNeasy Blood and Tissue Kit	150 CpG loci	methylation	bisulphite sequencing RainDrop BS-seq UroMark microdroplet PCR Taq polymerase		AUC: 97% NPV: 97%	98%	97%	107	167	London, United Kingdom	51.509865	-0.118092	Feber et al., 2017	
13	bladder cancer	Bladder Cancer	urine								centrifuged, pellet washed with PBS buffer DNeasy Blood and Tissue Kit	150 CpG loci	methylation	bisulphite sequencing RainDrop BS-seq UroMark microdroplet PCR Taq polymerase		AUC: 97% NPV: 97%	98%	97%	107	167	Birmingham, United Kingdom	52.489471	-1.898575	Feber et al., 2017	

14	urothelial carcinomas (UC)	Bladder Cancer	urine	50 mL							centrifuged, pellet washed twice with phosphate-buffered solution QIAamp DNA Mini Kit	VIM, CDH1, SALL3, TMEFF2, RASSF1A, BRCA1, GDF15, and ABCC6	methylation	bisulfite treatment EpiTect Fast Bisulfite Conversion Kits methylation-specific polymerase chain reaction (MSP) 2% agarose gel, ethidium bromide, UV positive control: methylated human genomic DNA	odds ratios for diagnosis: upper tract: univariable / multivariable CDH1: 21.106 / 16.354 GDF15: 0.257 / 0.238 HSPA2: 2.713 / 2.913 RASSF1A: 2.550 / 5.490 TMEFF2: not significant / 2.864 VIM: 4.343 / 5.078 muscle invasive: uni / multi GDF15: 0.214 / 0.214 high grade: uni / multi ABCC6: 0.274 / 0.367 CDH1: 6.439 / 4.678	chi-square tests logistic regression analyses p < 0.05 area under the receiver operating characteristic (ROC) curve (AUC)	VIM, CDH1, SALL3, TMEFF2, RASSF1A, BRCA1, GDF15, and ABCC6: 0.83	VIM, CDH1, SALL3, TMEFF2, RASSF1A, BRCA1, GDF15, and ABCC6: 0.60	217	256	Xi Cheng Qu, Bei Jing Shi, China	39.858425	116.287148	Guo et al., 2018	All biomarkers: ABCC6, BRCA1, CDH1, GDF15, HSPA2, RASSF1A, SALL3, THBS1, TMEFF2, and VIM
14	urothelial carcinomas (UC)	Bladder Cancer	urine								centrifuged, pellet washed twice with phosphate-buffered solution QIAamp DNA Mini Kit	CDH1, HSPA2, RASSF1A, TMEFF2, VIM, and GDF15	methylation	bisulfite treatment EpiTect Fast Bisulfite Conversion Kits methylation-specific polymerase chain reaction (MSP) 2% agarose gel, ethidium bromide, UV positive control: methylated human genomic DNA	odds ratios for diagnosis: upper tract: univariable / multivariable CDH1: 21.106 / 16.354 GDF15: 0.257 / 0.238 HSPA2: 2.713 / 2.913 RASSF1A: 2.550 / 5.490	chi-square tests logistic regression analyses p < 0.05 area under the receiver operating characteristic (ROC) curve (AUC)	CDH1, HSPA2, RASSF1A, TMEFF2, VIM, and GDF15: 0.82	CDH1, HSPA2, RASSF1A, TMEFF2, VIM, and GDF15: 0.68	217	256	Xi Cheng Qu, Bei Jing Shi, China	39.858425	116.287148	Guo et al., 2018	All biomarkers: ABCC6, BRCA1, CDH1, GDF15, HSPA2, RASSF1A, SALL3, THBS1, TMEFF2, and VIM

															TMEFF2: not significant / 2.864 VIM: 4.343 / 5.078 muscle invasive: uni / multi GDF15: 0.214 / 0.214 high grade: uni / multi ABCC6: 0.274 / 0.367 CDH1: 6.439 / 4.678											
14	urothelial carcinomas (UC)	Bladder Cancer	urine								centrifuged, pellet washed twice with phosphate-buffered solution QIAamp DNA Mini Kit	VIM, RASSF1A, GDF15, and TMEFF2	methylation	bisulfite treatment EpiTect Fast Bisulfite Conversion Kits methylation-specific polymerase chain reaction (MSP) 2% agarose gel, ethidium bromide, UV positive control: methylated human genomic DNA	odds ratios for diagnosis: upper tract: univariable / multivariable CDH1: 21.106 / 16.354 GDF15: 0.257 / 0.238 HSPA2: 2.713 / 2.913 RASSF1A: 2.550 / 5.490 TMEFF2: not significant / 2.864 VIM: 4.343 / 5.078 muscle invasive: uni / multi GDF15: 0.214 / 0.214 high grade: uni / multi ABCC6: 0.274 / 0.367 CDH1:	chi-square tests logistic regression analyses $p < 0.05$ area under the receiver operating characteristic (ROC) curve (AUC)	VIM, RASSF1A, GDF15, and TMEFF2: 0.82	VIM, RASSF1A, GDF15, and TMEFF2: 0.53	217	256	Xi Cheng Qu, Bei Jing Shi, China	39.858425	116.287148	Guo et al., 2018	All biomarkers: ABCC6, BRCA1, CDH1, GDF15, HSPA2, RASSF1A, SALL3, THBS1, TMEFF2, and VIM

															6.439 / 4.678											
14	urothelial carcinomas (UC)	Bladder Cancer	urine								centrifuged, pellet washed twice with phosphate-buffered solution QIAamp DNA Mini Kit	CDH1, SALL3, THBS1, MEFF2, VIM, and GDF15: 0.89, and with cytology	methylation	bisulfite treatment EpiTect Fast Bisulfite Conversion Kits methylation-specific polymerase chain reaction (MSP) 2% agarose gel, ethidium bromide, UV positive control: methylated human genomic DNA	odds ratios for diagnosis: upper tract: univariable / multivariable CDH1: 21.106 / 16.354 GDF15: 0.257 / 0.238 HSPA2: 2.713 / 2.913 RASSF1A: 2.550 / 5.490 TMEFF2: not significant / 2.864 VIM: 4.343 / 5.078 muscle invasive: uni / multi GDF15: 0.214 / 0.214 high grade: uni / multi ABCC6: 0.274 / 0.367 CDH1: 6.439 / 4.678	chi-square tests logistic regression analyses p < 0.05 area under the receiver operating characteristic (ROC) curve (AUC)	CDH1, SALL3, THBS1, MEFF2, VIM, and GDF15: 0.89, and with cytology: 0.91	CDH1, SALL3, THBS1, TMEFF2, VIM, and GDF15: 0.74, and with cytology: 0.92	217	256	Xi Cheng Qu, Bei Jing Shi, China	39.858425	116.287148	Guo et al., 2018	All biomarkers: ABCC6, BRCA1, CDH1, GDF15, HSPA2, RASSF1A, SALL3, THBS1, TMEFF2, and VIM
15	urothelial cell carcinoma	Bladder Cancer	urine							(not shared for urine)		VGF	methylation	EpiTect Bisulfite Kit PCR Taq Polymerase		Fisher's exact test p = 0.020	40%	Control: 5%	20	20	Baltimore, Maryland	39.299236	-76.609383	Hayashi et al., 2014	
16	bladder cancer	Bladder Cancer	urine	10-100 mL			0.02		0.0004	20 ng/µl	centrifuged, pellet suspended in phosphate-buffered saline QiAamp DNA micro-kit	TWIST1 and NID2	methylation	qPCR MethyLight assay	odds ratio for detection multivariate cancer: NID2: 1.06 TWIST1: 1.25 age: 1.04 grade:	Kruskal-Wallis and Fisher's exact test logistic regression models p < 0.05 area under the receiver	cancer: NID2: 76.8% TWIST1: 70.8% both NID2 & TWIST1: 76.2% grade: NID2: 77.8%	cancer: NID2: 61.56%, PPV: 81.46%, NPV: 55.96% TWIST1: 85.4%, PPV: 92.12, NPV: 58.03%	212	102	Toronto, Canada	43.65107	-79.347015	Hermanns et al., 2020	All biomarkers: TWIST1, RUNX3, GATA4, NID2, and FOXE1

															TWIST1: 1.04	operating characteristics curves (AUC).	TWIST1: 70.8% both NID2 & TWIST1: 77.6%	both NID2 & TWIST1: 83.33% grade: NID2: 60.2%, PPV: 81.37%, NPV: 58.31% TWIST1: 85.3%, PPV: 90.86, NPV: 56.94% both NID2 & TWIST1: 61.1%							
16	bladder cancer	Bladder Cancer	urine								centrifuged, pellet suspended in phosphate-buffered saline QiAamp DNA micro-kit	TWIST1 and NID2	methylation	qPCR MethyLight assay	odds ratio for detection multivariate cancer: NID2: 1.06 TWIST1: 1.25 age: 1.04 grade: TWIST1: 1.04	Kruskal-Wallis and Fisher's exact test logistic regression models $p < 0.05$ area under the receiver operating characteristics curves (AUC).	cancer: NID2: 76.8% TWIST1: 70.8% both NID2 & TWIST1: 76.2% grade: NID2: 77.8% TWIST1: 70.8% both NID2 & TWIST1: 77.6%	cancer: NID2: 61.56%, PPV: 81.46%, NPV: 55.96% TWIST1: 85.4%, PPV: 92.12, NPV: 58.03% both NID2 & TWIST1: 83.33% grade: NID2: 60.2%, PPV: 81.37%, NPV: 58.31% TWIST1: 85.3%, PPV: 90.86, NPV: 56.94% both NID2 & TWIST1: 61.1%	212	102	Halifax, Canada	44.65107	-63.582687	Hermanns et al., 2020	All biomarkers: TWIST1, RUNX3, GATA4, NID2, and FOXE1
16	bladder cancer	Bladder Cancer	urine								centrifuged, pellet suspended in phosphate-buffered saline QiAamp DNA micro-kit	TWIST1 and NID2	methylation	qPCR MethyLight assay	odds ratio for detection multivariate cancer: NID2: 1.06 TWIST1: 1.25	Kruskal-Wallis and Fisher's exact test logistic regression models $p < 0.05$	cancer: NID2: 76.8% TWIST1: 70.8% both NID2 & TWIST1: 76.2%	cancer: NID2: 61.56%, PPV: 81.46%, NPV: 55.96% TWIST1: 85.4%, PPV:	212	102	Zurich, Switzerland	47.373878	8.545094	Hermanns et al., 2020	All biomarkers: TWIST1, RUNX3, GATA4, NID2, and FOXE1

															age: 1.04 grade: TWIST1: 1.04	area under the receiver operating characteristics curves (AUC).	grade: NID2: 77.8% TWIST1: 70.8% both NID2 & TWIST1: 77.6%	92.12, NPV: 58.03% both NID2 & TWIST1: 83.33% grade: NID2: 60.2%, PPV: 81.37%, NPV: 58.31% TWIST1: 85.3%, PPV: 90.86, NPV: 56.94% both NID2 & TWIST1: 61.1%							
17	cervical cancer	Cervical Cancer	urine	10-50 mL			0.02		0.0007	at least 20 ng/μL	EZNA Tissue DNA Extraction kit	SFRP4 and ZAR1 promoters	methylation	DNA integrity quantified with 268-base pair fragment of β-globin (HBB) bisulfite modification EZ DNA Methylation Kit-GoldTM quantitative methylation-specific PCR (QMSP) Taq Polymerase	SFRP4: non-lesion (mean = 0) L-SIL (mean = 0.04098) H-SIL (mean = 0.07513) SCC (mean = 4.98) ZAR1: not significant differences between groups	chi-square test Fisher's exact test Kruskal Wallis test and Dunn's post-test Cohen's kappa coefficient ROC AUC: 0.633 p<0.005	45.16%	83.30%	40 low-grade squamous intraepithelial lesions (L-SIL) 40 high-grade squamous intraepithelial lesions (H-SIL) 31 cervical squamous cell carcinoma (SCC)	60 non-lesions	Temuco, Chile	-38.73965	-72.59842	Hoffstetter et al., 2017	
18	colorectal cancer	Colorectal Cancer	feces			<10 copies DNA						NDRG4 and BMP3	methylation	multitarget stool DNA test		a logistic-regression algorithm positive threshold: 183 p<0.001	cancer: 92.3%	nonadvanced or negative findings: 86.6%	65 colorectal patients	9167 nonadvanced or negative findings	United States	38.889722	-77.008889	Imperiale et al., 2014	90 sites in U.S. and Canada
18	colorectal cancer	Colorectal Cancer	feces									NDRG4 and BMP3	methylation	multitarget stool DNA test		a logistic-regression algorithm positive threshold: 183 p<0.001	cancer: 92.3%	nonadvanced or negative findings: 86.6%	65 colorectal patients	9167 nonadvanced or negative findings	Canada	56.130366	-106.346771	Imperiale et al., 2014	

18	colorectal cancer	Colorectal Cancer	feces									NDRG4 and BMP3	methylation	multitarget stool DNA test		a logistic-regression algorithm positive threshold: 183 p<0.001	polyps with high grade dysplasia: 69.2% serrated sessile polyps: 42.4%	nonadvanced or negative findings: 86.6%	757 with advanced adenomas or polyps	9167 nonadvanced or negative findings	United States	38.889722	-77.008889	Imperiale et al., 2014	
18	colorectal cancer	Colorectal Cancer	feces									NDRG4 and BMP3	methylation	multitarget stool DNA test		a logistic-regression algorithm positive threshold: 183 p<0.001	polyps with high grade dysplasia: 69.2% serrated sessile polyps: 42.4%	nonadvanced or negative findings: 86.6%	757 with advanced adenomas or polyps	9167 nonadvanced or negative findings	Canada	56.130366	-106.346771	Imperiale et al., 2014	
19	urothelial carcinoma	Bladder Cancer	urine			at least 10 copies of the control ACTB gene						TWIST1 and NID2	methylation	methylation-specific polymerase chain reaction (MSPCR) reference gene: β-Actin		logistic regression model AUC of ROC 95% CI	79% optimized: 75%	63% optimized: 71%	111		Durham, North Carolina	35.994034	-78.898621	Abern et al., 2014	
20	bladder cancer	Bladder Cancer	urine				0.5			maximum 500 ng	centrifuged, pellet washed in PBS filtered Qiagen Mini Prep kit concentration analysis: NanoDrop spectrophotometer	BCL2, EOMES, HOXA9, VIM2, CCNA1, POU4F2, and SALL3	methylation	bisulfite treatment EZ DNA MethylationGold Kit Real-time quantitative methylation-specific PCR MethyLight reference gene: ALUC4 positive control: bisulfite treated Human Genomic DNA pyrosequencing assays			75%		189	11	Herlev, Denmark	55.72876	12.43728	Andersson et al., 2014	
21	Non–Small Cell Lung Cancer	Lung Cancer	urine	10 mL							centrifuged, preserved in EDTA Proteinase K, Buffer AL isopropanol, Magnetic Beads	CDO1, TAC1, HOXA7, HOXA9, SOX17, and ZFP42 promoters	methylation	methylation on beads (MOB) protocol bisulfite conversionmethylation-specific PCR (qMSP) reference gene: β-Actin	Concordance: 78.3%	95% CI Wilcoxon rank sum test Fisher exact test Pearson correlation analysis	methylation in 3 or more genes: 73%	methylation in 3 or more genes: 92%	74	27	Baltimore, Maryland	39.299236	-76.609383	B. Liu et al., 2020	
21	Non–Small Cell Lung Cancer	Lung Cancer	urine								centrifuged, preserved in EDTA Proteinase K, Buffer AL isopropanol, Magnetic Beads	CDO1, TAC1, HOXA7, HOXA9, SOX17, and ZFP42	methylation	methylation on beads (MOB) protocol bisulfite conversionmethylation-specific PCR	Concordance: 78.3%	95% CI Wilcoxon rank sum test Fisher exact test	methylation in 3 or more genes: 73%	methylation in 3 or more genes: 92%	74	27	Chicago, Illinois	41.881832	-87.623177	B. Liu et al., 2020	

												promoters		(qMSP) reference gene: β-Actin		Pearson correlation analysis								
22	colorectal cancer	Colorectal Cancer	urine				0.4			up to 400 ng DNA	centrifuged, pellet or unfractionated analyzed Quick DNA urine kit concentration analysis: Qubit™ dsDNA HS Assay	SEPT9, TMEFF2, SDC2, NDRG4, VIM and ALX4	methylation	bisulfite conversion EZ DNA Methylation kit quantitative Methylation Specifc PCRs Epitect Multiplex PCR Mastermix reference gene: ACTB	SEPT9 and SDC2: 70% cases	Mann Whitney U test Pearson's Chi-square test p v<0.05, Bonferroni correction Kruskal Wallis test multivariate logistic regression Receiver Operator Characteristic (ROC) curve		SEPT9 and SDC2: 86%	92	63	Amsterdam, The Netherlands	52.377956	4.89707	Bach et al., 2021	
23	kidney cancer	Kidney Cancer	urine	200–500 µl			0.25		1.0000	0.25–1 µg DNA	centrifuged, pellet analyzed proteinase K phenol/chloroform extraction ethanol precipitation	VHL, p16/CDKN2a, p14ARF, APC, RASSF1A, and Timp-3	methylation	bisulfite treatment methylation-specific PCR		Fisher's exact test P ≤ 0.05	88%	100%	50	12 healthy individuals 9 nephrolithiasis (renal stones) 3 benign renal cysts	Philadelphia, Pennsylvania	39.952583	-75.165222	Battagli et al., 2003	
24	bladder cancer	Bladder Cancer	urine	2 to 40ml			0.05		0.0026	50ng DNA	Quick-DNA™ Urine Kit	CADM1, FAM19A4, GHSR, MAL, PHACTR3, PRDM14, SST, ZIC1, miR-124-2, miR-129, miR-137, miR-148, miR-181 and miR-935	methylation	bisulfite treatment EZ DNA Methylation™ kit quantitative methylation-specific PCR reference gene: ACTB		Mann-WhitneyUtest chi-square test Multivariate logistic regression analysis 95% CI	92%	85%	72	75	Amsterdam, The Netherlands	52.377956	4.89707	Bosschieter et al., 2019	
25	bladder cancer	Bladder Cancer	urine					4		4uL DNA	Tissue and Cell Genomic DNA Purification Kit	DAPK, IRF8, p14, RASSF1A and SFRP1	methylation	bisulfite treatment EZ DNA Methylation Kit quantitative MSP Taq Polymerase		Kruskal-Wallis Test Mann-Whitney test chi-square test Fisher's	86.70%	94.70%	30	19	Taiwan, China	23.69781	120.96051	Chen et al., 2011	

														unmethylated positive control: human peripheral lymphocyte DNA		exact test AUC of ROC Kaplan-Meier analysis log-rank test P < 0.05										
25	bladder cancer	Bladder Cancer	urine								Tissue and Cell Genomic DNA Purification Kit	DAPK, IRF8, p14, RASSF1A and SFRP1	methylation	bisulfite treatment EZ DNA Methylation Kit quantitative MSP Taq Polymerase unmethylated positive control: human peripheral lymphocyte DNA		Kruskal-Wallis Test Mann-Whitney test chi-square test Fisher's exact test AUC of ROC Kaplan-Meier analysis log-rank test P < 0.05	86.70%	94.70%	30	19	Hong Kong, China	22.302711	114.177216	Chen et al., 2011	
26	bladder cancer	Bladder Cancer	urine	20 mL			0.5		0.0250	<500 ng of urinary cfDNA	centrifugation filtration	cfDNA	methylation copy number	bisulfite conversion EpiTect Bisulfite Kit KAPA HTP Library Preparation Kit Shallow-depth paired-end genome-wide bisulfite sequencing		Mann–Whitney U-test 95% CI	Methylomic and copy number: 93.5%	Methylomic and copy number: 95.8%	46	39	Hong Kong, China	22.302711	114.177216	Cheng et al., 2019	
27	urothelial cancer	Bladder Cancer	urine	50 mL			2		0.0400	2 ug DNA	centrifuged, pellet washed in PBS	hyper: SOX1, TJP2, MYOD, HOXA9_1, HOXA9_2 hypo: VAMP8, CASP8, SPP1, IFNG, CAPG, HLADPA1, RIPK3	methylation	bisulfite treatment Epitect Bisulfite Kit (Qiagen) bisulfite pyrosequencing GoTaq Hot Start Polymerase		Pearson correlation coefficient Mann–Whitney test AUC of ROC	100%	100%	73	18	Los Angeles, California	34.052235	-118.243683	Chihara et al., 2013	

27	urothelial cancer	Bladder Cancer	urine								centrifuged, pellet washed in PBS	hyper: SOX1, TJP2, MYOD, HOXA9_1, HOXA9_2 hypo: VAMP8, CASP8, SPP1, IFNG, CAPG, HLADPA1, RIPK3	methylation	bisulfite treatment Epitect Bisulfite Kit (Qiagen) bisulfite pyrosequencing GoTaq Hot Start Polymerase		Pearson correlation coefficient Mann–Whitney test AUC of ROC	100%	100%	73	18	Nara, Japan	34.68505	135.80485	Chihara et al., 2013	
27	urothelial cancer	Bladder Cancer	urine								centrifuged, pellet washed in PBS	hyper: SOX1, TJP2, MYOD, HOXA9_1, HOXA9_2 hypo: VAMP8, CASP8, SPP1, IFNG, CAPG, HLADPA1, RIPK3	methylation	bisulfite treatment Epitect Bisulfite Kit (Qiagen) bisulfite pyrosequencing GoTaq Hot Start Polymerase		Pearson correlation coefficient Mann–Whitney test AUC of ROC	100%	100%	73	18	Tokyo, Japan	35.652832	139.839478	Chihara et al., 2013	
27	urothelial cancer	Bladder Cancer	urine								centrifuged, pellet washed in PBS	hyper: SOX1, TJP2, MYOD, HOXA9_1, HOXA9_2 hypo: VAMP8, CASP8, SPP1, IFNG, CAPG, HLADPA1, RIPK3	methylation	bisulfite treatment Epitect Bisulfite Kit (Qiagen) bisulfite pyrosequencing GoTaq Hot Start Polymerase		Pearson correlation coefficient Mann–Whitney test AUC of ROC	100%	100%	73	18	Tochigi, Japan	36.455254	139.804108	Chihara et al., 2013	
28	prostate cancer	Prostate Cancer	urine	30 mL	4.5 mL		0.015		0.0005	15-20 ng RNA	centrifuge, pellet analyzed concentration analysis: NanoString	GSTP1, SFRP2, IGFBP3, IGFBP7, APC, and PTSG2	methylation	quantitative methylation-specific polymerase chain reaction (qPCR)	proportional odds ratio = 2.04 per 0.1 ExoMeth increase Gleason ≥3 + 4: AUC 0.89 Gleason≥4 + 3: AUC 0.81	logistic regression 95% CI	90% accuracy		197		Dublin, Ireland	53.35014	-6.266155	Connell et al., 2020	

28	prostate cancer	Prostate Cancer	urine								centrifuge, pellet analyzed concentration analysis: NanoString	GSTP1, SFRP2, IGFBP3, IGFBP7, APC, and PTSG2	methylation	quantitative methylation-specific polymerase chain reaction (qPCR)	proportional odds ratio = 2.04 per 0.1 ExoMeth increase Gleason ≥3 + 4: AUC 0.89 Gleason≥4 + 3: AUC 0.81	logistic regression 95% CI	90% accuracy		197		Toronto, Ontario, Canada	43.65107	-79.347015	Connell et al., 2020	
28	prostate cancer	Prostate Cancer	urine								centrifuge, pellet analyzed concentration analysis: NanoString	GSTP1, SFRP2, IGFBP3, IGFBP7, APC, and PTSG2	methylation	quantitative methylation-specific polymerase chain reaction (qPCR)	proportional odds ratio = 2.04 per 0.1 ExoMeth increase Gleason ≥3 + 4: AUC 0.89 Gleason≥4 + 3: AUC 0.81	logistic regression 95% CI	90% accuracy		197		Norwich, Norfolk, United Kingdom	52.630886	1.297355	Connell et al., 2020	
29	bladder cancer	Bladder Cancer	urine	20–50 mL			1		0.0286	at least 1 ug DNA	centrifuged, pellet washed in phosphate-buffered saline phenol-chloroform procedure	TCF21 and PCDH17 promoters	methylation	bisulfite treatment real-time quantitative methylation-specific polymerase chain reaction (qMSP) AmpliTaq Gold reference gene: ACTB		Kruskall-Wallis test Mann-Whitney U-test Kruskall-Wallis tests Spearman nonparametric correlation test Receiver operator characteristics (ROC) curve, area under the curve (AUC) P < 0.05	bladder 60% overall: 39%	100%	50 bladder cancer	48 healthy individuals	Manassas, Virginia	38.75066	-77.475143	Costa et al., 2011	
29	bladder cancer	Bladder Cancer	urine								centrifuged, pellet washed in phosphate-buffered saline phenol-chloroform procedure	TCF21 and PCDH17 promoters	methylation	bisulfite treatment real-time quantitative methylation-specific polymerase chain reaction (qMSP) AmpliTaq Gold reference gene: ACTB		Kruskall-Wallis test Mann-Whitney U-test Kruskall-Wallis tests Spearman nonparametric correlation test	renal: 32% overall: 39%	100%	50 renal cancer	48 healthy individuals	Manassas, Virginia	38.75066	-77.475143	Costa et al., 2011	

																Receiver operator characteristics (ROC) curve, area under the curve (AUC) P < 0.05									
29	bladder cancer	Bladder Cancer	urine								centrifuged, pellet washed in phosphate-buffered saline phenol-chloroform procedure	TCF21 and PCDH17 promoters	methylation	bisulfite treatment real-time quantitative methylation-specific polymerase chain reaction (qMSP) AmpliTaq Gold reference gene: ACTB		Kruskall-Wallis test Mann-Whitney U-test Kruskall-Wallis tests Spearman nonparametric correlation test Receiver operator characteristics (ROC) curve, area under the curve (AUC) P < 0.05	prostate: 26% overall: 39%	100%	50 prostate cancer	48 healthy individuals	Manassas, Virginia	38.75066	-77.475143	Costa et al., 2011	
30	bladder cancer	Bladder Cancer	urine	30–60 mL							centrifuged, pellet washed in phosphate-buffered saline QIAamp DNA Mini Kit	RAR-β2	methylation	bisulfite treatment EZ DNA Methylation-Gold™ kit methylation specific nested PCR puReTaq Polymerase 2% agarose gel, ethidium bromide positive control: Universal methylated human liver DNA standard		Kruskal–Wallis Mann–Whitney U non-parametric tests chi-square test ROC, AUC P < 0.05	65%	89.70%	100	65 benign urological disorders 51 healthy volunteers	Cairo, Egypt	30.033333	31.233334	Eissa et al., 2012	
31	bladder cancer	Bladder Cancer	urine					18		18 µl of DNA	centrifuged, pellet analyzed	DAPK, BCL-2, and H-TERT	methylation	bisulfite treatment real-time quantitative methylation-sensitive PCR (MethyLight)		Kaplan-Meier analysis log-rank test Kaplan-Meier curve	81.10%	100%	37	20	Hamburg, Germany	53.551086	9.993682	Friedrich et al., 2007	

														control: Sss1-treated DNA methylated with methylase		P < 0.05									
32	colorectal cancer	Colorectal Cancer	feces	4 g			2			2 ug DNA	centrifuged, RNase A sodium acetate-isopropanol, ethanol wash,TE proteinase K phenol/chloroform/isoamyl alcohol, LoTE QIAamp DNA stool mini kit	TFPI2	methylation	bisulfite modifed Zymo Kit Quantitative methylation-specific PCR Taq polymerase		Receiver operator characteristic (ROC) curve analysis, area under the curve	CRC: 76% PPV: 94%	CRC: 93% NPV: 73%	47 CRC	30	Amsterdam, The Netherlands	52.377956	4.897007	Glöckner et al., 2009	
32	colorectal cancer	Colorectal Cancer	feces								centrifuged, RNase A sodium acetate-isopropanol, ethanol wash,TE proteinase K phenol/chloroform/isoamyl alcohol, LoTE QIAamp DNA stool mini kit	TFPI2	methylation	bisulfite modifed Zymo Kit Quantitative methylation-specific PCR Taq polymerase		Receiver operator characteristic (ROC) curve analysis, area under the curve	adenomas: 21% PPV: 67%	adenomas: 93% NPV: 65%	19 adenomas	30	Amsterdam, The Netherlands	52.377956	4.897007	Glöckner et al., 2009	
33	colorectal cancer	Colorectal Cancer	feces	4 g			2			2 ug DNA		GATA4 and GATA5 promoters	methylation	Quantitative MSP (qMSP) Taq polymerase		Pearson's chi-square test Fisher's exact test and the one-way ANOVA Kruskal-Wallis Mann-Whitney test Mc Nemar test paired t-test Receiver operator characteristic (ROC) curve, area under the curve (AUC) 95% CI	51%	93%	75	75	Maastricht, The Netherlands	50.851368	5.690973	Hellebrekers et al., 2009	
34	kidney cancer	Kidney Cancer	urine	15 mL			2		0.1333	2 ug DNA	centrifuged, pellet analyzed proteinase K phenol/chloroform extraction ethanol precipitation	CDH1 promoter	methylation	bisulfite treatement quantitative fluorogenic real-time PCR TaqMan	at least one gene methylated: 88%		Clinical: CDH1: 38%	CDH1: 95%	17 primary kidney cancer 9 renal cancer	91 without genitourinary cancer	Baltimore, Maryland	39.299236	-76.609383	Hoque et al., 2004	

34	kidney cancer	Kidney Cancer	urine								centrifuged, pellet analyzed proteinase K phenol/chloroform extraction ethanol precipitation	APC promoter	methylation	bisulfite treatement quantitative fluorogenic real-time PCR TaqMan	at least one gene methylated: 88%		Clinical: APC: 38%	APC: 96%	17 primary kidney cancer 9 renal cancer	91 without genitourinary cancer	Baltimore, Maryland	39.299236	-76.609383	Hoque et al., 2004	
34	kidney cancer	Kidney Cancer	urine								centrifuged, pellet analyzed proteinase K phenol/chloroform extraction ethanol precipitation	MGMT promoter	methylation	bisulfite treatement quantitative fluorogenic real-time PCR TaqMan	at least one gene methylated: 88%		Clinical: MGMT: 8%	MGMT: 100%	17 primary kidney cancer 9 renal cancer	91 without genitourinary cancer	Baltimore, Maryland	39.299236	-76.609383	Hoque et al., 2004	
34	kidney cancer	Kidney Cancer	urine								centrifuged, pellet analyzed proteinase K phenol/chloroform extraction ethanol precipitation	RASSF1A promoter	methylation	bisulfite treatement quantitative fluorogenic real-time PCR TaqMan	at least one gene methylated: 88%		Clinical: RASSF1A: 65%	RASSF1A: 89%	17 primary kidney cancer 9 renal cancer	91 without genitourinary cancer	Baltimore, Maryland	39.299236	-76.609383	Hoque et al., 2004	
34	kidney cancer	Kidney Cancer	urine								centrifuged, pellet analyzed proteinase K phenol/chloroform extraction ethanol precipitation	GSTP1 promoter	methylation	bisulfite treatement quantitative fluorogenic real-time PCR TaqMan	at least one gene methylated: 88%		Clinical: GSTP1: 15%	GSTP1: 100%	17 primary kidney cancer 9 renal cancer	91 without genitourinary cancer	Baltimore, Maryland	39.299236	-76.609383	Hoque et al., 2004	
34	kidney cancer	Kidney Cancer	urine								centrifuged, pellet analyzed proteinase K phenol/chloroform extraction ethanol precipitation	p16 promoter	methylation	bisulfite treatement quantitative fluorogenic real-time PCR TaqMan	at least one gene methylated: 88%		Clinical: p16: 35%	p16: 100%	17 primary kidney cancer 9 renal cancer	91 without genitourinary cancer	Baltimore, Maryland	39.299236	-76.609383	Hoque et al., 2004	
34	kidney cancer	Kidney Cancer	urine								centrifuged, pellet analyzed proteinase K phenol/chloroform extraction ethanol precipitation	RAR-β2 promoter	methylation	bisulfite treatement quantitative fluorogenic real-time PCR TaqMan	at least one gene methylated: 88%		Clinical: RAR-β2: 31%	RAR-β2: 91%	17 primary kidney cancer 9 renal cancer	91 without genitourinary cancer	Baltimore, Maryland	39.299236	-76.609383	Hoque et al., 2004	
34	kidney cancer	Kidney Cancer	urine								centrifuged, pellet analyzed proteinase K phenol/chloroform extraction ethanol precipitation	ARF promoter	methylation	bisulfite treatement quantitative fluorogenic real-time PCR TaqMan	at least one gene methylated: 88%		Clinical: ARF: 31%	ARF: 100%	17 primary kidney cancer 9 renal cancer	91 without genitourinary cancer	Baltimore, Maryland	39.299236	-76.609383	Hoque et al., 2004	
34	kidney cancer	Kidney Cancer	urine								centrifuged, pellet analyzed proteinase K phenol/chloroform extraction ethanol precipitation	TIMP3 promoter	methylation	bisulfite treatement quantitative fluorogenic real-time PCR TaqMan	at least one gene methylated: 88%		Clinical: TIMP3: 46%	TIMP3: 91%	17 primary kidney cancer 9 renal cancer	91 without genitourinary cancer	Baltimore, Maryland	39.299236	-76.609383	Hoque et al., 2004	
35	bladder cancer	Bladder Cancer	urine	15 mL			2		0.1333	2 ug DNA	centrifuged, pellet washed in phosphate-buffered saline proteinase K phenol/chloroform, ethanol wash	APC, ARF, CDH1, GSTP1, MGMT, CDKN2A, RARβ2, RASSF1	methylation	bisulfite treatment Wizard DNA Clean-Up System fluorescence-based real-		logistic regression model AUC of ROC 95% CI	69%	at least one methylated gene: 10%	160	69	Baltimore, Maryland	39.299236	-76.609383	Hoque et al., 2006	

												A, and TIMP3 promoters		time PCR Taq polymerase reference gene: ACTB												
36	colorectal cancer	Colorectal Cancer	feces	50 g				10		10 uL	DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM and HLTF	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	72.50%	86.90%	125	200	New York, New York	40.73061	-73.935242	Itzkowitz et al., 2007	
36	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM and HLTF	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	72.50%	86.90%	125	200	Evanston, Illinois	42.045597	-87.688568	Itzkowitz et al., 2007	
36	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM and HLTF	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	72.50%	86.90%	125	200	Ontario, Canada	50	-85	Itzkowitz et al., 2007	
36	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM and HLTF	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	72.50%	86.90%	125	200	Boston, Massachusetts	42.361145	-71.057083	Itzkowitz et al., 2007	
36	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM and HLTF	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	72.50%	86.90%	125	200	Hines, Illinois	41.85216	-87.837	Itzkowitz et al., 2007	
36	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM and HLTF	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	72.50%	86.90%	125	200	Norfolk, Virginia	36.850769	-76.285873	Itzkowitz et al., 2007	
36	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM and HLTF	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	72.50%	86.90%	125	200	Marlborough, Massachusetts	42.345463	-71.551628	Itzkowitz et al., 2007	

36	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM and HLTF	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	72.50%	86.90%	125	200	Cleveland, Ohio	41.505493	-81.68129	Itzkowitz et al., 2007	
37	colorectal cancer	Colorectal Cancer	feces					10		10 µL	DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	hV: 83% hypermethylated vimentin gene (hV) and a two site DNA integrity assay (DY): 86%	hV: 82% DY: 85% hV + DY: 73%	40	122	New York, New York	40.73061	-73.935242	Itzkowitz et al., 2008	
37	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	hV: 83% hypermethylated vimentin gene (hV) and a two site DNA integrity assay (DY): 86%	hV: 82% DY: 85% hV + DY: 73%	40	122	Evanston, Illinois	42.045597	-87.688568	Itzkowitz et al., 2008	
37	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	hV: 83% hypermethylated vimentin gene (hV) and a two site DNA integrity assay (DY): 86%	hV: 82% DY: 85% hV + DY: 73%	40	122	Ontario, Canada	50	-85	Itzkowitz et al., 2008	
37	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	hV: 83% hypermethylated vimentin gene (hV) and a two site DNA integrity assay (DY): 86%	hV: 82% DY: 85% hV + DY: 73%	40	122	Boston, Massachusetts	42.361145	-71.057083	Itzkowitz et al., 2008	
37	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	hV: 83% hypermethylated vimentin gene (hV) and a two site DNA integrity assay (DY): 86%	hV: 82% DY: 85% hV + DY: 73%	40	122	Hines, Illinois	41.85216	-87.837	Itzkowitz et al., 2008	
37	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM	methylation	bisulfite conversion MSP PCR HotStar Polymerase		t tests chi-square test P < 0.05	hV: 83% hypermethylated vimentin gene (hV) and a two site DNA	hV: 82% DY: 85% hV + DY: 73%	40	122	Norfolk, Virginia	36.850769	-76.285873	Itzkowitz et al., 2008	

														4% NuSieve 3:1 agarose			integrity assay (DY): 86%								
37	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	hV: 83% hypermethylated vimentin gene (hV) and a two site DNA integrity assay (DY): 86%	hV: 82% DY: 85% hV + DY: 73%	40	122	Marlborough, Massachusetts	42.345463	-71.551628	Itzkowitz et al., 2008	
37	colorectal cancer	Colorectal Cancer	feces								DNA-stabilizing buffer Integrity assay using real-time polymerase chain reaction (PCR	VIM	methylation	bisulfite conversion MSP PCR HotStar Polymerase 4% NuSieve 3:1 agarose		t tests chi-square test P < 0.05	hV: 83% hypermethylated vimentin gene (hV) and a two site DNA integrity assay (DY): 86%	hV: 82% DY: 85% hV + DY: 73%	40	122	Cleveland, Ohio	41.505493	-81.68129	Itzkowitz et al., 2008	
38	prostate cancer	Prostate Cancer	urine				0.5			0.5 ug DNA	centrifuged, pellet purified IBI kit DNeasy Blood & Tissue Kit	PLA2G16 CpG adjacent loci	methylation	EpiTect Plus FFPE Bisulfite Kit one primer-biotinylated PCR streptavidin sepharose beads pyrosequencing assay 2% agarose gel	0.8 predictive value 1.37 odds ratio	95% CI probability cutoff: 0.065	92%	35%	90 tumor-associated 77 non-tumor associated	12	Madison, Wisconsin	43.073051	-89.40123	Jarrard et al., 2019	
39	low-risk prostate cancer	Prostate Cancer	urine	5–40 ml								GSTP1 and APC	methylation	internal control: β-Actin external control: targeted plasma DNA 2-step PCR assay RT–PCR		logistic prediction model logistic regression model p-value Student's t test area under the receiver operating characteristic curve (AUC) Wilcoxon–Mann–Whitney test statistic	Gleason score of 7 or greater PPV: 100%	Gleason score of 7 or greater NPV: 100%	biopsy cohort: 243 prostatectomy cohort: 99 males	biopsy cohort (no prostate cancer diagnoses): 430	18 clinics: United States	38.889722	-77.008889	Jatkoe et al., 2015	
40	bladder cancer	Bladder Cancer	urine								proteinase K digestion protocol	p14ARF, RUNX3, RARβ, DAPK,	methylation	methylation-specific polymerase chain reaction	all 3: AUC 0.936 RUNX3 and	chi-square test AUC of	all3: 98.21% RUNX3 and	all3: 88.89% RUNX3 and	112	10 healthy individuals	Changchun, Jilin, China	43.891262	125.331345	K. Wang et al., 2016	

											concentration analysis: NanoDrop	and HPP1		GC buffer PCR Master Mix 1% agarose gel	RARβ: AUC 0.918 p14ARF and HPP1: AUC 0.688	ROC P < 0.05	RARβ: 96.64% p14ARF and HPP1: 37.50%	RARβ: 88.89% p14ARF and HPP1: 100%		17 glandular cystitis patients					
41	bladder cancer	Bladder Cancer	urine				0.5			0.5 µg DNA		TBX2	methylation	differential methylation hybridization protocol Illumina PCR validation	predictors of progresion: TBX2 predicting progression: area under the curve of 0.96 , p < 0.0001 TBX3: p = 0.04 GATA2: p = 0.03	p < 0.05 Benjamini and Hochberg method Kaplan-Meier method multivariate Cox regression	TBX2: 100% positive predictive value: 78%	TBX2: 80% negative predictive value: 100%	4		Rotterdam, The Netherlands	51.926517	4.462456	Kandimalla et al., 2012	All biomarkers: TBX2, TBX3, GATA2, and ZIC4 CpG Island
42	bladder cancer	Bladder Cancer	urine	25–100 mL			0.02		0.0003	20 ng DNA	centrifuged, pellet washed in PBS DNeasy Tissue kit	OTX1, MEIS1, ONECUT2, SIM2, FOXA1, ZNF503, HOXA9, and OSR1	methylation	bisulfite treatment EZ DNA methylation gold kit		logistic regression model AUC of ROC 95% CI	74%	90%	95		Aarhus, Denmark	56.156635	10.210365	Kandimalla et al., 2013	
43	colon cancer	Colorectal Cancer	feces	4 g						32 ug DNA (outlier)	stool stabilization buffer stool homogenization buffer centrifuged, pellet submerged in RNAse A sodium acetate & isopropanol and ethanol wash DNA pellet re-suspended in TE & proteinase K digested with SDS incubation, centrifugation phenol/chloroform/isoamylalcohol, incubation, centrifugation DNA precipitated, washed Pellets re-suspended in TE solution	OSMR CpGs	methylation	EZ-96DNA Methylation Kit Clean and Concentrator Kit Sequencing and Combined Bisulfite Restriction Analysis (COBRA) PCR gel, UV SYBR Green Gold		p < 0.01 receiver operating characteristic (ROC) curves Z values (AUROC0.5)/Std Err (standard error of AUROC)	38% Stage I: 11% Stage II: 56% Stage III: 44% Stage IV: 17%	Controls: 5%	69	81	Tilman, Belgium	50.59058	5.571054	Kim et al., 2009	
44	prostate cancer	Prostate Cancer	urine	20 to 600 mL		more than 30 copies/uL DNA in sodium bisulfite					filtered Nucleopore track etched polycarbonate membrane filter centrfuge, pellet suspended in lysis/stabilization buffer	GSTP1, APC, RASSF1A, PITX2 and C1orf114p	methylation	bisulfite treatment EZ DNA Methylation-Gold Kit droplet digital polymerase chain reaction	prostate specific antigen and urine: AUC 0.95	Welch t-test Spearman rank order correlation 95% CI	digital rectal examination before: 81% after: 60%		117	12	Herlev, Denmark	55.72876	12.43728	Larsen et al., 2018	Journal of Urology

														ddPCR Supermix control: MYOD1										
45	colorectal cancer	Colorectal Cancer	feces	250 mg			2			2 ug DNA	DNA: QIAamp DNA Stool Mini Kit RNA: QIAamp Viral Mini Spin Kit	APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1 COX-2 mRNA	methylation expression	bisulfite treatment EZ DNA Methylation Kit methylation-specific PCR positive control: universal methylated DNA RT-PCR Reverse Trascriptase		Fisher's exact test Student's t test 95% CI	colorectal: 75%	90%	20 Colorectal patients	30	Hong Kong, China	22.302711	114.177216	Leung et al., 2007	
45	colorectal cancer	Colorectal Cancer	feces								DNA: QIAamp DNA Stool Mini Kit RNA: QIAamp Viral Mini Spin Kit	APC, ATM, hMLH1, sFRP2, HLTF, MGMT, and GSTP1 COX-2 mRNA	methylation expression	bisulfite treatment EZ DNA Methylation Kit methylation-specific PCR positive control: universal methylated DNA RT-PCR Reverse Trascriptase		Fisher's exact test Student's t test 95% CI	adenomas: 68%	90%	30 paients with polyps	30	Hong Kong, China	22.302711	114.177216	Leung et al., 2007	
46	colorectal cancer	Colorectal Cancer	feces			200 or more copies of b-actin					sDNA stabilization buffer target-specific magnetic beads	BMP3 and NDRG4	methylation	enzyme-linked immunosorbent assay plates			98% stage I: 95% stage II: 100% stage III: 96% stage IV: 100% advanced precancers (AA and SSA) ≥1 cm: 57% >2 cm: 73% >3 cm: 83%	90%	93 CRC 84 advanced adenomas 30 sessile serrated adenoma	155 nonadvanced adenomas 641 no colonic lesions	Rochester, Minnesota	44.016369	-92.475395	Lidgard et al., 2013	
46	colorectal cancer	Colorectal Cancer	feces								sDNA stabilization buffer target-specific magnetic beads	BMP3 and NDRG4	methylation	enzyme-linked immunosorbent assay plates			98% stage I: 95% stage II: 100% stage III: 96%	90%	93 CRC 84 advanced adenomas 30 sessile	155 nonadvanced adenomas 641 no colonic lesions	Scottsdale, Arizona	33.501324	-111.925278	Lidgard et al., 2013	

																	stage IV: 100% advanced precancers (AA and SSA) ≥1 cm: 57% >2 cm: 73% >3 cm: 83%		serrated adenoma						
47	bladder cancer	Bladder Cancer	urine				4			4 ug DNA	proteinase K phenol/chloroform	E-cadherin, p16, p14, and RASSF1A	methylation	bisulfite treatment Methylation-specific PCR (MSP) assay positive control: Lymphocyte DNA methylated with SssI methylase		chi-square test k test P < 0.05	83%	100%	57	20	Kaohsiung, Taiwan	22.633333	120.26667	Lin et al., 2010	
48	colorectal cancer and adenomas	Colorectal Cancer	feces					1		1 uL of bisulfite-modified DNA		CNRIP1, FBN1, INA, MAL, and SNCA promoters	methylation	bisulfite treatment TaqMan Universal PCR master mix NoAmpErase UNG Reference gene: ALU-C4		Pearson's chi-square test Fisher's exact test Student T-test Mann-Whitney U test Receiver Operating Characteristics (ROC) curves					South-East Norway	59.911491	10.757933	Lind et al., 2011	unclear which data is stool-specific
49	colorectal cancer	Colorectal Cancer	feces					1			Total RNA extraction Qiagen miRNAeasy Mini Kits concentration analysis: RiboGreen RNA quantitation kits	miR-21 and miR-106a	expression	Qiagen miRNAeasy Mini Kit SAM-Bead microarray Illumina qPCR TaqMan Polymerase or SYBR Green	ΔCt ± SD miR-21: colonic adenomas: 7.6 ± 1.6 CRC: 6.9 ± 0.5 healthy: 6.1 ± 1.6 miR-106a: colonic adenomas: 0.5 ± 1.6 CRC: −0.2 ± 0.5 healthy: 0.6 ± 1.6	Student's t tests ANOVA or Kruskall-Wallis Spearman's or Pearson's test logarithmic regression P < 0.05			29	8	Dallas, Texas	32.779167	-96.808891	Link et al., 2010	
50	low grade papillary urothelial cell carcinom	Bladder Cancer	urine					2		2 µL of bisulfite-modified DNA	phenol-chloroform extraction protocol	CCND2 promoter	methylation	EpiTect Bisulfite kit fluorescence-based real-time PCR	LGPUCC: recurrent: CCND2 53% CCNA1: 58%	cross tabulations and chi-square test Fisher's	UCC cases CCND2: 26%	controls CCND2: 0%	148	56	Baltimore, Maryland	39.299236	-76.609383	Maldonado et al., 2014	All biomarkers: ARF, TIMP3, RAR-β2, NID2,

	a (LGPUCC)													Taq polymerase reference gene: β-actin	NID2: 68% CALCA: 53% nonrecurrent: CCND2: 12% CCNA1: 23.5% NID2: 18% CALCA: 23.5%	exact test Student t-test									CCNA1, AIM1, CALCA and CCND2 promoters
50	low grade papillary urothelial cell carcinoma (LGPUCC)	Bladder Cancer	urine								phenol-chloroform extraction protocol	CCNA1 promoter	methylation	EpiTect Bisulfite kit fluorescence-based real-time PCR Taq polymerase reference gene: β-actin	LGPUCC: recurrent: CCND2 53% CCNA1: 58% NID2: 68% CALCA: 53% nonrecurrent: CCND2: 12% CCNA1: 23.5% NID2: 18% CALCA: 23.5%	cross tabulations and chi-square test Fisher's exact test Student t-test	UCC cases CCNA1: 68%	controls CCNA1: 17%	148	56	Baltimore, Maryland	39.299236	-76.609383	Maldonado et al., 2014	All biomarkers: ARF, TIMP3, RAR-β2, NID2, CCNA1, AIM1, CALCA and CCND2 promoters
50	low grade papillary urothelial cell carcinoma (LGPUCC)	Bladder Cancer	urine								phenol-chloroform extraction protocol	CALCA promoter	methylation	EpiTect Bisulfite kit fluorescence-based real-time PCR Taq polymerase reference gene: β-actin	LGPUCC: recurrent: CCND2 53% CCNA1: 58% NID2: 68% CALCA: 53% nonrecurrent: CCND2: 12% CCNA1: 23.5% NID2: 18% CALCA: 23.5%	cross tabulations and chi-square test Fisher's exact test Student t-test	UCC cases CALCA: 63.5%	controls CALCA: 28.5%	148	56	Baltimore, Maryland	39.299236	-76.609383	Maldonado et al., 2014	All biomarkers: ARF, TIMP3, RAR-β2, NID2, CCNA1, AIM1, CALCA and CCND2 promoters
51	colorectal cancer	Colorectal Cancer	feces				2			2 ug DNA		EN1, SCTR, INHBB CpG Islands	methylation	bisulfite treatment Real-time PCR temperature dissociation (melting curve (MC)) Fast Start DNA Master Sybr Green I mix		t-tests or analysis of variance (ANOVA) chi-square or Fisher's-exact test Kaplan – Meier method	44% positive patients 27% overall	97%	30		Barcelona, Spain	41.390205	2.154007	Mayor et al., 2009	
52	bladder cancer	Bladder Cancer	urine	20 mL			0.1		0.0050	at least 100 ng DNA	ZR Urine DNA Isolation Kit validated: Nanodrop	RBBP8	methylation	bisulfite conversion EZ DNA Methylation™ kit		p < 0.05 maximum likelihood estimator non-	RBBP8: 52%	RBBP8: 91%	52	13	Aachen, Germany	50.775555	6.083611	Mijnes et al., 2018	

														positive control: EpiTect® PCR Control DNA Set qRT-PCR SYBR Green		parametric Mann-Whitney U test Dunn's multiple comparison n test Fisher's exact test Kaplan-Meier method Multivariate Cox regression analysis Receiver operating characteristics (ROC)									
53	upper-tract urothelial carcinomas	Bladder Cancer	urine					2		2 µL of bisulphite modified DNA	centrifuged, pellet washed in phosphate-buffered saline phenol–chloroform validated: Nanodrop	GDF15, TMEFF2 and VIM promoter	methylation	bisulfite modified EZ DNA methylation – Gold kit methylation-specific polymerase chain reaction (MSP) TaqMan Universal PCR Master Mix No AmpErase UNG positive control: in vitro methylated human DNA		non-parametric Mann–Whitney U-test Mann–Whitney and Kruskall–Wallis tests, Spearman non-parametric correlation test receiver operator characteristics (ROC) curve, area under de curve (AUC) Kaplan–Meier with log rank tes Cox-regression model P < 0.05, with Bonferroni's correction	91%	100%	22	20	Porto, Portugal	41.150223	-8.629932	Monteiro-Reis et al., 2014	
54	prostate cancer	Prostate Cancer	urine	20-30 mL				3		3 µL of bisulphite	centrifuged, pellet washed in PBS phenolchloroform	singleplex-miR-34b/c and miR-	methylation	bisulfite sodium conversion EZ DNA Methylation-		Kruskal-Wallis and Mann-Whitney	singleplex-miR-34b/c and miR-193b:	singleplex-miR-34b/c and miR-	87	32	Porto, Portugal	41.150223	-8.629932	Moreira-Barbosa et al., 2018	

										modified DNA		193b; multiplex -APC, GSTP1, and RARβ2 promoters		Gold™ Kit singleplex: KAPA SYBR FAST qPCR Kit multiplex: MethyLight assay reference gene: β-Actin		non-parametric tests P < 0.05, Bonferroni's correction Spearman non-parametric correlation test receiver operator characteristics (ROC) curves, area under the curve (AUC) logistic regression models Kaplan-Meier with log rank test Cox-regression model	95.4% PPV: 94.3% multiplex-APC, GSTP1, and RARβ2: 94.3% PPV: 94.3%	193b: 84.4% NPV: 87.1% multiplex -APC, GSTP1, and RARβ2: 84.4% NPV: 84.4%							
55	renal cell carcinoma (RCC)	Kidney Cancer	urine	2 mL			0.001		0.0005	1 ng DNA	centrifuged, pellet Qiagen genomic DNA extraction Kit proteinase K, AE buffer concentration analysis Qubit dsDNA High Sensitivity Assay Kit	Differentially Methylated Regions (DMRs) 300 DMRs: 150 DMRs with gain in RCC &150 DMRs with loss in RCC	methylation	cfMeDIP–seq protocol KAPA HyperPrep Kit AMPure XP beads MagMeDIP kit high-throughput sequencing KAPA HiFi Hotstart ReadyMix NEBNext Multiplex Oligos for Illumina	cell-free DNA: AUROC 0.86						Boston, Massachusetts	42.361145	-71.057083	Nuzzo et al., 2020	
56	aggressive prostate cancer	Prostate Cancer	urine	≤ 50 mL								GSTP1, SFRP2, IGFBP3, IGFBP7, APC, and PTGS2	methylation	quantitaive methylation-specific PCR epiCaPture	area under the curve PCa: 0.64 high-grade PCa: 0.86 high-risk PCa: 0.83 epiCaPture and PSA: 0.82	P < .05 logistic regression models area under the receiver operating characteristic curve (AUC)	epiCaPture and PSA: 0.73	epiCaPture and PSA: 0.76	244	209	Atlanta, Georgia	33.753746	-84.38633	O'Reilly et al., 2019	

																decision curve									
56	aggressive prostate cancer	Prostate Cancer	urine									GSTP1, SFRP2, IGFBP3, IGFBP7, APC, and PTGS2	methylation	quantitaive methylation-specific PCR epiCaPture	area under the curve PCa: 0.64 high-grade PCa: 0.86 high-risk PCa: 0.83 epiCaPture and PSA: 0.82	P < .05 logistic regression models area under the receiver operating characteristic curve (AUC) decision curve	epiCaPture and PSA: 0.73	epiCaPture and PSA: 0.76	244	209	Toronto, Ontario, Canada	43.65107	-79.347015	O'Reilly et al., 2019	
56	aggressive prostate cancer	Prostate Cancer	urine									GSTP1, SFRP2, IGFBP3, IGFBP7, APC, and PTGS2	methylation	quantitaive methylation-specific PCR epiCaPture	area under the curve PCa: 0.64 high-grade PCa: 0.86 high-risk PCa: 0.83 epiCaPture and PSA: 0.82	P < .05 logistic regression models area under the receiver operating characteristic curve (AUC) decision curve	epiCaPture and PSA: 0.73	epiCaPture and PSA: 0.76	244	209	Dublin, Ireland	53.35014	-6.266155	O'Reilly et al., 2019	
56	aggressive prostate cancer	Prostate Cancer	urine									GSTP1, SFRP2, IGFBP3, IGFBP7, APC, and PTGS2	methylation	quantitaive methylation-specific PCR epiCaPture	area under the curve PCa: 0.64 high-grade PCa: 0.86 high-risk PCa: 0.83 epiCaPture and PSA: 0.82	P < .05 logistic regression models area under the receiver operating characteristic curve (AUC) decision curve	epiCaPture and PSA: 0.73	epiCaPture and PSA: 0.76	244	209	Norwich, Norfolk, England	52.630886	1.297355	O'Reilly et al., 2019	
57	colorectal cancer	Colorectal Cancer	feces	10 g	0.2 g		0.02			20 ng of bisulfite-modified DNA	washed PBS homogenized, EDTA buffer centrifuged, pellet submerged in lysis buffers proteinase K phenol-chloroform-isoamylalcohol sodium acetate, isopropanol ethanol wash, TE buffer QIAamp DNA Stool Mini Kit concentration	SDC2	methylation	bisulfite conversion EZ DNA Methylation-Gold kit purification Zymo-Spin IC column 2-rounds PCR unidirectional linear target enrichment & quantitative methylation-specific real time PCR (LTE-qMSP)	AUC 0.933	95% CI area under the receiver operating characteristic curve (AUC)	90% stage I: 83.3% II: 88.2% III: 90.0% IV: 100%	90.9%%	CRC: 50 pre-cancerous lesions: 21	healthy individuals: 22	Seoul, South Korea	37.5326	127.024612	Oh et al., 2017	

											analysis Qubit dsDNA BR assay kit			AptaTaq PCR master mix control: COL2A1 region without CpGs											
57	colorectal cancer	Colorectal Cancer	feces								washed PBS homogenized, EDTA buffer centrifuged, pellet submerged in lysis buffers proteinase K phenol-chloroform-isoamylalcohol sodium acetate, isopropanol ethanol wash, TE buffer QIAamp DNA Stool Mini Kit concentration analysis Qubit dsDNA BR assay kit	SDC2	methylation	bisulfite conversion EZ DNA Methylation-Gold kit purification Zymo-Spin IC column 2-rounds PCR unidirectional linear target enrichment & quantitative methylation-specific real time PCR (LTE-qMSP) AptaTaq PCR master mix control: COL2A1 region without CpGs	AUC 0.933	95% CI area under the receiver operating characteristic curve (AUC)	90% stage I: 83.3% II: 88.2% III: 90.0% IV: 100%	90.9%%	CRC: 50 pre-cancerous lesions: 21	healthy individuals: 22	Ilsan, South Korea	38.183333	127.816667	Oh et al., 2017	
58	renal cell carcinoma (RCC)	Kidney Cancer	urine	4 mL				8		8 uL DNA	cohort 2: centrifuged, washed in PBS phenol-chloroform	microRNA-30a-5p: two CpGs	methylation	bisulfite conversion EZ DNA Methylation-Gold™ Kit cohort 3: SsoAdvanced™ PreAmp Supermix quantitative Methylation-specific PCR (qMSP) Xpert Fast SYBR reference gene: β-Actin		receiver operator characteristics (ROC) Kaplan Meier curves (with log rank)	testing: 83% validation: 63%	testing: 53% validation: 67%	cohort 2: 53 cohort 3: 171	cohort 2: 57 cohort 3: 85	Porto, Portugal	41.150223	-8.629932	Outeiro-Pinho et al., 2020	
59	urothelial carcinoma	Bladder Cancer	urine								phenol-chloroform protocol concentration analysis NanoDrop	miR-129-2 and miR-663a promoters	methylation	sodium bisulphite conversion EZ DNA Methylation-Gold Kit real-time quantitative methylationspecific PCR		receiver operator characteristics curve (ROC) area under the curve (AUC) Mann–	87.70%	84%	49	25 prostate cancer 25 renal cancer 25 healthy individuals	Porto, Portugal	41.150223	-8.629932	Padra˜o et al., 2017	

														KAPA SYBR FAST qPCR Kit reference gene: ACTB		Whitney U test Spearman ’s method, Mann–Whitney or Kruskall–Wallis tests DeLong’s test McNemar proportion test Kaplan–Meier with log rank test A Cox regression model P < 0.05, Bonferron ´s correction									
60	colorectal cancer	Colorectal Cancer	feces								QIAamp DNA Stool Mini Kit	VIM	methylation	bisulfite conversion EpiTect® Fast DNA Bisulfite Kit quantitative methylation-specific PCR internal control: ACTB		t-test or ANOVA Fisher’s exact test P < 0.05	60% PPV: 100%	100%	49	30	Mashhad, Iran	36.310699	59.599457	Pakbaz et al., 2019	
61	systemic lupus erythematosus (SLE)	Systemic Lupus Erythematosus (SLE)	urine								(CFN) cell-free: centrifuge, pellet analyzed RNA: mirVana PARIS kit (Exo) pellet containing exosomes: protease inhibitor cocktail RNA: Total exosome RNA and protein isolation kit (Sn) exosome-depleted supernatant: unltracentrifuged, pellet analyzed RNA: mirVana PARIS kit concentration analysis: NanoDrop	miR-335, miR-302d, miR-200c and miR-146a	microRNA expression	quantitative reverse-transcription PCR TaqMan microRNA assay and Reverse Transcription kit	Exo significantly increased for all miRNAs compared to Sn fraction, especially miR146a (6-fold change, p<0.01)	Student’s t-test Fisher’s exact test Mann–Whitney U-tes AUC of ROC P < 0.05			38	12	Valencia, Spain	39.466667	-0.375	Perez-Hernandez et al., 2015	
62	colorectal cancer	Colorectal Cancer	feces							4 mg/mL DNA in RNAse (outlier)	centrifuged, pellet washed in PBS TNE, RNase sodium acetate,	CDKN2A, MGMT, and MLH1	methylation	sodium bisulfite treatment	adenomas: CDKN2A: 31%	t tests chi-square tests Fisher			66		Nashville, Tennessee	36.174465	-86.76796	Petka et al., 2005	

											isopropal alcohol, ethanol wash			Methylation Specific PCR 3% agarose gel, ethidium bromide, UV positive controls: DNA from peripheral blood leukocytes, DNA extracted from colorectal cancer cell lines known to be methylated for the three target genes	MGMT: 48% MLH1: 0% no polyps: CDKN2A: 16% MGMT: 27% MLH1: 10%	exact tests									
63	bladder cancer	Bladder Cancer	urine				0.005			5 ng of urine DNA	Bladder CARE Urine Collection Kit Quick-DNA™ Urine Kit Femto™ Human DNA Quantification Kit 5 ng DNA or more	3 BC-specific genes 2 internal control genes	methyl ation	bisulfite-FREE Bladder CARE	LOD as low as 0.046% "detecting 1 cancer cell for every 2,200 cells analyzed"	Student's two-tailed t tests. receiver operating characteristic (ROC) curve area under the curve (AUC)	93.5% PPV: 87.8%	92.6% NPV: 96.2%	77	136	Los Angeles, California	34.05 2235	-118.24 3683	Piatti et al., 2021	
64	bladder cancer	Bladder Cancer	urine								centrifuged, pellet in PBS QIAamp DNA Mini Kit	p14ARF, p16INK4A, RASSF1A, DAPK, and APC promoters	methyl ation	bisulfite conversion EpiTect Bisulfite kit MSP EpiTect Whole Bisulfitome Kit vs. UroVysion test interphase nuclei, cell-by-cell basis 20 target cells, nuclear size or irregular nuclear shape FISH pattern	p14ARF 32% p16INK4a 12% RASSF1A 46% DAPK1 17% APC 46%	Pearson's chi-square test Fisher's exact test p value < 0.05	91%		117	8	Bełchatów, Poland	51.36 883	19.356 71	Pietrusiński et al., 2017	
65	colorectal cancer	Colorectal Cancer	feces								Stool Nucleic Acid Collection and Preservation Tubes Stool Total RNA Purification Kit	miR-486-5p	microRNA expression	NEBNext® Multiplex Small RNA Library Prep Set for Illumina small-RNA-Seq pipeline analyses	CRC: 223.67 reads control: 43.65 reads	likelihood ratio test (LRT) Benjamini–Hochberg (BH)-adjusted p value < 0.05 Jonckheere–Terpstra test			CRC: 58	healthy individuals: 79	Sassari, Italy	40.72 5925	8.5556 83	Pisano et al., 2020	

66	prostate cancer	Prostate Cancer	urine				0.3			300 ng DNA		miR-130a promoter	methylation	EZ DNA Methylation Gold Kit quantitative methylation-specific PCR (qMSP)	AUC: 0.89		83.50%	82.30%	101	15	Porto, Portugal	41.150223	-8.629932	Ramalho-Carvalho et al., 2017	
67	bladder cancer	Bladder Cancer	urine				0.5			500 ng of RNA	Puregene DNA Purification Kit concentration analysis: OD260/280 ratio	ZNF154, POU4F2, HOXA9, and EOMES	methylation	PCR-based MS-HRM technique		Pearson correlation coefficient Mann–Whitney test 95% CI	84%	96%	115	59 benign	Aarhus, Denmark	56.156635	10.210365	Reinert et al., 2011	
68	bladder cancer	Bladder Cancer	urine				0.005			5 ng of bisulfite-modified DNA	QIAsymphony Virus/Bacteria Midi kit	EOMES, HOXA9, POU4F2, TWIST1, VIM, and ZNF154	methylation	bisulfite modified EZ-96 DNA methylation D5004 real-time methylation-specific PCR (MethyLight) TaqMan Universal PCR Master Mix No AmpErase positive control: Universal Methylated DNA) with ALU-C4		Wilcoxon-Mann-Whitney test Fisher's exact test chi-square exact test Spearman correlation coefficients ROC curve, area under the curve (AUC) Kaplan-Meier survival plots Univariate Cox regression analysis P < 0.05	validation: 82%-89% recurrence: 88%-94%	validation: 94%-100% recurrence: 43%-67%	184	35	Aarhus, Denmark	56.156635	10.210365	Reinert et al., 2012	
69	bladder cancer	Bladder Cancer	urine	50 mL			1.5		0.0300	up to 1.5 µg of DNA	concentration analysis: Quant-iT PicoGreen dsDNA Quantitation Kit	TWIST1 and NID2	methylation	bisulfite conversion MSP normalization gene: β-Actin		logistic regression model AUC of ROC	90% PPV: 86%	93% NPV: 95%	33	57	Lie`ge, Belgium	50.63373	5.56749	Renard et al., 2009	
70	non-muscle-invasive bladder cancer (NMIBC)	Bladder Cancer	urine				0.05			50 ng DNA	nylon membrane filter wash phosphate-buffered saline QiAmp DNA Mini kit AL lysis buffer proteinase K	HS3ST2, SLIT2 and SEPTIN9	methylation	sodium bisulfite treatment EZ DNA Methylation kit quantitative multiplex methylation-specific PCR (QMMSP) TaqMan Polymerase	optimism-corrected AUC: 0.96 follow-up: AUC: 0.84 threshold algorithm: AUC: 0.82	logistic regression models area under the receiver operating characteristic curve (AUC)	97.6% follow-up: 90.3% threshold algorithm: 94.5%	84.8 % NPV: 99.6% follow-up: 65.1% NPV: 97.0% threshold algorithm: 75.9%	167	105	Paris, France	48.864716	2.349014	Roperch et al., 2016	

														normalization: ALB with no CpGs				NPV: 98.5%							
71	prostate cancer	Prostate Cancer	urine								ultracentrifugation PBS independent set: exosomal RNA purification procedure	urinary extracellular vesicles (uEVs) CMTM3 and CDH3 transcripts	particle number independent set: expression	Nanoparticle-tracking analysis independent set: qRTPCR reference genes: GAPDH and RPL6		Pearson Correlation test Student T test Mann Whitney U test	normalization against RPL6: 0.69 p = 0.055 normalization against GAPDH: 0.65 p = 0.01				Biscay, Spain	43.326702	-2.990057	Royo et al., 2015	
72	bladder cancer	Bladder Cancer	urine	15 mL				1		1 µl extracted DNA	phenol/chloroform ethanol precipitation	BCL2, CDKN2A and NID2	methylation	bisulfite treatment EpiTect® Bisulfite Kit nested methylation specific polymerase chain reaction assay HotStart Taq polymerase		Fisher's exact test	80.90%	86.40%	42	21	Holon, Israel	32.01034	34.77918	Scher et al., 2012	Journal of Urology
73	bladder cancer	Bladder Cancer	urine	20-500 mL			2		0.0077	2 ug DNA	centrifuge, pellet washed in phosphate-buffered saline QIAamp DNA Mini Kit	APC, RASSF1A and SFRP2	methylation	bisulfite conversion reference gene: ACTB MethyLight TaqMan assay reference gene: ALUC4		Fisher's exact test Exact chi-square test Pearson correlation coefficient	52% APC, RASSF1A and SFRP2: 62%	APC, RASSF1A and SFRP2: 100%	113	33 noncancerous urinary lesions	Herlev, Denmark	55.72876	12.43728	Serizawa et al., 2011	All biomarkers: APC, ARF, DBC1, INK4A, RARB, RASSF1A, SFRP1, SFRP2, SFRP4, SFRP5 and WIF1
74	bladder cancer	Bladder Cancer	urine	10 mL			0.005		0.0005	5 ng of total RNA	centrifuge, pellet analyzed phenol-chloroform	miR-137, miR-124-2, miR-124-3, and miR-9-3	methylation	Bisulfite conversion methylation-specific PCR (MSP) bisulfite sequencing, pyrosequencing Reverse transcription Quantitative real-time polymerase chain TaqMan Polymerase	AUC: 0.916	Student t test one-way analysis of variance (ANOVA), post hoc Tukey test Fisher exact test Pearson correlation coefficient Receiver operating characteristic (ROC) curve p < 0.05	81%	89%	86	20	Hokkaido, Japan	43.066666	141.350006	Shimizu et al., 2013	
75	colorectal cancer	Colorectal Cancer	feces				0.5			500 ng of bisulfite-	microbiota: GNOME DNA Isolation Kit	16s rRNA	microbiota sequen	microbiota: PCR Illumina		Pearson's chi-square test	cohort 1: 29.2%	cohort 1: 89.7%	cohort 1: 226 cohort 2:	cohort 2: 500 without	Paris, France	48.864716	2.349014	Sobhani et al., 2019	

										converted DNA	human: QiAamp DNA stool Mini Kit	NPY, Wif1, and NPY	cing human methylation	sequencing human: bisulfite conversion EZ DNA Methylation kit Illumina Golden Gate methylation bead arrays quantitative, single-gene methylation-specific PCR (QS-MSP) quantitative multiplex methylation-specific PCR (QMMSP) reference gene: ALB without CpGs		Student's t test logistic regression model P < 0.05			500 digestive symptoms	digestive symptoms	Créteil, France				
76	non–muscle-invasive bladder cancer (NMIBC)	Bladder Cancer	urine	40 mL			0.5		0.0125	≤ 500 ng	centrifuged, pellet washed in phosphate-buffered saline phenol/chloroform/isoamyl alcohol concentration analysis: NanoDrop	DLX1, ITGA4, SOX17, ASTN1, RXFP3, and ZNF671	methylation	bisulfite treatment EpiTect Fast DNA Bisulfite Kit quantitative methylation-specific PCR (qMSP) GynTect		Fisher exact test Cohen's κ 95% CI	60%	96.70%	40	30	Jena, Germany	50.927223	11.586111	Steinbach et al., 2020	
77	bladder cancer	Bladder Cancer	urine	50 mL							centridufuged, pellet analyzed	SOX1, IRAK3, andL1-MET	methylation	bisulfite conversion EZ DNA Methylation Kit PCR biotin-labeled primers pyrosequencing		logistic regression models area under the receiver operating characteristic curve (AUC) 95% CI	SOX1, IRAK3, andL1-MET 3-gene panel: 86%	SOX1, IRAK3, andL1-MET 3-gene panel: 89%	20	20	Los Angeles, California	34.052235	-118.243683	Su et al., 2014	All biomarkers: HOXA9, SOX1, NPY, IRAK3, and ZO2, and hypomethylation of L1-MET
78	bladder cancer	Bladder Cancer	urine				10			10 ug DNA	proteinase K/organic extraction method	SFRP1	methylation	bisulfite conversion real-time PCR		Fisher's exact test AUC of ROC 95% CI	SFRP1: 36.65%	SFRP1: 93.3%	82	15	Shanghai, China	31.224361	121.46917	Sun et al., 2009	
79	colorectal cancer	Colorectal Cancer	fecal occult blood								samples mixed in buffer solution hemoglobin > 0.2 μg/ml	SEPT9	methylation	Immune colloidal gold technique		ANOVA Spearman's rank correlations t tests area under the	CRC patients: 73.0%% PPV: 63.0% intestinal polyps and	CRC patients: 94.5% NPV: 96.5% intestinal polyps and	650		Tianjin , China	39.133331	117.183334	Sun et al., 2019	

																curve (AUC) of the receiver operating characteristic curve (ROC) Fisher exact test Logistic regression analysis Chi-square tests P < 0.05	adenoma: 17.1% PPV: 34.1%	adenoma : 94.5% NPV: 87.3%							
80	non–muscle-invasive bladder cancer (NMIBC)	Bladder Cancer	urine	12 mL							Cytolyt fixation liquid centrifuged, pellet analyzed Bladder EpiCheck DNA extraction kit	15 (proprietary)	methylation	quantitative real-time polymerase chain reaction (qRT-PCR) Bladder EpiCheck test		Pearson chi-square test AUC of ROC z test P <0.05	62.3% low-grade tumors: 46.1% high-grade tumors: 83.3% cytology and EpiCheck: 66.7% low-grade tumors: 56.4% high-grade tumors: 90% PPV: 68.2% cytology and EpiCheck: 68.6%	86.3% cytology and EpiCheck: 85.6% NPV: 82.9% cytology and EpiCheck: 84.5%	243		Bolzano, Italy	46.5	11.35	Trenti et al., 2019	
81	bladder cancer	Bladder Cancer	urine				1			1 ug Total RNA	centrifuged, pellet purified QIAamp DNA Mini Kit	sFRP-1	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test	sFRP-1: 75.9%	sFRP-1: 53.7%	24	20	San Francisco, California	37.773972	-122.431297	Urakami et al., 2006	

																Fisher's exact test P < 0.05										
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	sFRP-1	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05	sFRP-1: 75.9%	sFRP-1: 53.7%	24	20	Izumo, Japan	35.36667	132.76667	Urakami et al., 2006	
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	sFRP-1	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05	sFRP-1: 75.9%	sFRP-1: 53.7%	24	20	Kagoshima, Japan	30.732412	30.732412	Urakami et al., 2006	
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	sFRP-2	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684	logistic regression models area under the receiver operator characteristics (ROC) curve	sFRP-2: 63.0%	sFRP-2: 57.4%	24	20	San Francisco, California	37.773972	-122.431297	Urakami et al., 2006	

														control: GADPH	Wif-1: 0.669	(AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05										
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	sFRP-2	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05	sFRP-2: 63.0%	sFRP-2: 57.4%	24	20	Izumo, Japan	35.36667	132.76667	Urakami et al., 2006	
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	sFRP-2	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05	sFRP-2: 63.0%	sFRP-2: 57.4%	24	20	Kagoshima, Japan	30.732412	30.732412	Urakami et al., 2006	
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified	sFRP-4	methylation	bisulfite treatment	AUC: sFRP-1: 0.683	logistic regression models	sFRP-4: 38.9%	sFRP-4: 87.0%	24	20	San Francisc	37.773972	-122.431297	Urakami et al., 2006	

											QIAamp DNA Mini Kit			methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05					o, California				
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	sFRP-4	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05	sFRP-4: 38.9%	sFRP-4: 87.0%	24	20	Izumo, Japan	35.3667	132.7667	Urakami et al., 2006	
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	sFRP-4	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square	sFRP-4: 38.9%	sFRP-4: 87.0%	24	20	Kagoshima, Japan	30.732412	30.732412	Urakami et al., 2006	

																test Fisher's exact test P < 0.05										
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	sFRP-5	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05	sFRP-5: 87.0%	sFRP-5: 37.0%	24	20	San Francisco, California	37.773972	-122.431297	Urakami et al., 2006	
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	sFRP-5	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05	sFRP-5: 87.0%	sFRP-5: 37.0%	24	20	Izumo, Japan	35.36667	132.76667	Urakami et al., 2006	
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	sFRP-5	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684	logistic regression models area under the receiver operator characteristics (ROC)	sFRP-5: 87.0%	sFRP-5: 37.0%	24	20	Kagoshima, Japan	30.732412	30.732412	Urakami et al., 2006	

														internal control: GADPH	Wif-1: 0.669	curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05									
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	Wif-1	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05	Wif-1: 63.0%	Wif-1: 72.2%	24	20	San Francisco, California	37.773972	-122.431297	Urakami et al., 2006	
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	Wif-1	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05	Wif-1: 63.0%	Wif-1: 72.2%	24	20	Izumo, Japan	35.366667	132.766667	Urakami et al., 2006	

81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	Wif-1	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05	Wif-1: 63.0%	Wif-1: 72.2%	24	20	Kagoshima, Japan	30.732412	30.732412	Urakami et al., 2006	
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	Dkk-3	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05	Dkk-3: 38.9%	Dkk-3: 92.6%	24	20	San Francisco, California	37.773972	-122.431297	Urakami et al., 2006	
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	Dkk-3	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test	Dkk-3: 38.9%	Dkk-3: 92.6%	24	20	Izumo, Japan	35.366667	132.766667	Urakami et al., 2006	

																Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05										
81	bladder cancer	Bladder Cancer	urine								centrifuged, pellet purified QIAamp DNA Mini Kit	Dkk-3	methylation	bisulfite treatment methylation-specific PCR 2% agarose gel, UV bisulfite sequencing internal control: GADPH	AUC: sFRP-1: 0.683 sFRP-2: 0.611 sFRP-4: 0.614 sFRP-5: 0.620 Dkk-3: 0.684 Wif-1: 0.669	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) Mann-Whitney test Kruskal-Wallis test chi-square test Fisher's exact test P < 0.05	Dkk-3: 38.9%	Dkk-3: 92.6%	24	20	Kagoshima, Japan	30.732412	30.732412	Urakami et al., 2006	
82	endometrial cancer	Endometrial Cancer	urine		15 mL		0.05		0.0033	50 ng modified DNA	DNA mini and blood mini kit concentration analysis: NanoDrop	GHSR, SST, and ZIC1	methylation	bisulfte conversion EZ DNA Methylation Kit quantitative methylation-specifc PCR (qMSP) reference gene: ACTB	AUC full void urine: AUC 0.86 to 0.95 GHSR: 0.95 SST: 0.92 ZIC1: 0.86	Spearman's rank correlation Mann–Whitney U test receiver operating characteristic (ROC) area under the curve (AUC)			42	46	Amsterdam, The Netherlands	52.377956	4.89707	van den Helder et al., 2020	
83	bladder cancer	Bladder Cancer	urine				1			1 ug DNA	centrifuged, pellet analyzed QIAamp DNA Mini Kit concentration analysis: NanoDrop	CFTR, SALL3, and TWIST1	methylation	bisulfite modification EpiTect Bisulfite kit Ampli Taq Gold 360 DNA polymerase 2% agarose gel, ethidium bromide pyrosequencing biotin-labeled	methylation and cytology: CFTR, SALL3, and TWIST1: AUC 0.86	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) p < 0.1	methylation and cytology: CFTR, SALL3, and TWIST1: 96% PPV: 56%	methylation and cytology: CFTR, SALL3, and TWIST1: 40%, NPV: 92%	111	57	Barcelona, Spain	41.390205	2.154007	van der Heijden et al., 2018	All biomarkers: CDH13, CFTR, NID2, SALL3, TMEFF2, TWIST1, and VIM2
83	bladder cancer	Bladder Cancer	urine								centrifuged, pellet analyzed	CFTR, SALL3,	methylation	bisulfite modification EpiTect	methylation and cytology:	logistic regression models	methylation and cytology:	methylation and cytology:	111	57	Nijmegen, The	51.8425	5.85278	van der Heijde	All biomarkers: CDH13,

											QIAamp DNA Mini Kit concentration analysis: NanoDrop	and TWIST1		Bisulfite kit Ampli Taq Gold 360 DNA polymerase 2% agarose gel, ethidium bromide pyrosequencing biotin-labeled	CFTR, SALL3, and TWIST1: AUC 0.86	area under the receiver operator characteristics (ROC) curve (AUC) p < 0.1	CFTR, SALL3, and TWIST1: 96% PPV: 56%	CFTR, SALL3, and TWIST1: 40%, NPV: 92%			Netherlands			n et al., 2018	CFTR, NID2, SALL3, TMEFF2, TWIST1, and VIM2
83	bladder cancer	Bladder Cancer	urine								centrifuged, pellet analyzed QIAamp DNA Mini Kit concentration analysis: NanoDrop	CFTR, SALL3, and TWIST1	methylation	bisulfite modification EpiTect Bisulfite kit Ampli Taq Gold 360 DNA polymerase 2% agarose gel, ethidium bromide pyrosequencing biotin-labeled	methylation and cytology: CFTR, SALL3, and TWIST1: AUC 0.86	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) p < 0.1	methylation and cytology: CFTR, SALL3, and TWIST1: 96% PPV: 56%	methylation and cytology: CFTR, SALL3, and TWIST1: 40%, NPV: 92%	111	57	Bucharest, Romania	44.389122	26.115875	van der Heijden et al., 2018	All biomarkers: CDH13, CFTR, NID2, SALL3, TMEFF2, TWIST1, and VIM2
83	bladder cancer	Bladder Cancer	urine								centrifuged, pellet analyzed QIAamp DNA Mini Kit concentration analysis: NanoDrop	CFTR, SALL3, and TWIST1	methylation	bisulfite modification EpiTect Bisulfite kit Ampli Taq Gold 360 DNA polymerase 2% agarose gel, ethidium bromide pyrosequencing biotin-labeled	methylation and cytology: CFTR, SALL3, and TWIST1: AUC 0.86	logistic regression models area under the receiver operator characteristics (ROC) curve (AUC) p < 0.1	methylation and cytology: CFTR, SALL3, and TWIST1: 96% PPV: 56%	methylation and cytology: CFTR, SALL3, and TWIST1: 40%, NPV: 92%	111	57	Houston, Texas	29.749907	-95.358421	van der Heijden et al., 2018	All biomarkers: CDH13, CFTR, NID2, SALL3, TMEFF2, TWIST1, and VIM2
84	hematuria	Hematuria	urine				0.1			100 ng DNA	QIAamp DNA Micro Kit concentration analysis: Quant-iT PicoGreen dsDNA Quantification Kit	TWIST1, ONECUT2,and OTX1 methylation FGFR3, TERT and HRAS mutation	mutation methylation	bisulfite treatment EZ DNA Methylation-Lightning Kit TWIST1: Methylation specific polymerase chain reaction Quantitative PCR mix OTX1 and ONECUT2: SNaPshot™	TWIST1, ONECUT2 ,and OTX1 methylation & FGFR3, TERT and HRAS mutation panel: AUC 0.93 optimism corrected AUC 0.92.	95% CI	TWIST1, ONECUT2,and OTX1 methylation & FGFR3, TERT and HRAS mutation panel: 97%	TWIST1, ONECUT2,and OTX1 methylation & FGFR3, TERT and HRAS mutation panel: 83% NOV: 99.6-99.9%	74: bladder cancer	80 without bladder cancer	Rotterdam, The Netherlands	51.926517	4.462456	van Kessel et al., 2016	All biomarkers: TERT, PIK3CA, FGFR3, HRAS, KRAS and NRAS mutations OTX1, ONECUT2 and TWIST1 methylation
85	hematuria	Hematuria	urine								QIAmp DNA Mini-Kit	FGFR3, TERT and HRAS mutation OTX1,	mutation methylation	bisulfite treatment EZ DNA Methylation-Gold QuantiTect	AUC 0.96 optimism corrected AUC 0.95	logistic regression models Mann-Whitney U test Fisher	93%	86% NPV: 99%	97	103	Rotterdam, The Netherlands	51.926517	4.462456	van Kessel et al., 2017	

												ONECUT 2 and TWIST1 methylation		Multiplex PCR Master Mix		exact test area under the receiver operator characteristics (ROC) curve (AUC) 95% CI										
85	hematuria	Hematuria	urine								QIAmp DNA Mini-Kit	FGFR3, TERT and HRAS mutation OTX1, ONECUT 2 and TWIST1 methylation	mutation methylation	bisulfite treatment EZ DNA Methylation-Gold QuantiTect Multiplex PCR Master Mix	AUC 0.96 optimism corrected AUC 0.95	logistic regression models Mann-Whitney U test Fisher exact test area under the receiver operator characteristics (ROC) curve (AUC) 95% CI	93%	86% NPV: 99%	97	103	Barcelona, Spain	41.390205	2.154007	van Kessel et al., 2017	
85	hematuria	Hematuria	urine								QIAmp DNA Mini-Kit	FGFR3, TERT and HRAS mutation OTX1, ONECUT 2 and TWIST1 methylation	mutation methylation	bisulfite treatment EZ DNA Methylation-Gold QuantiTect Multiplex PCR Master Mix	AUC 0.96 optimism corrected AUC 0.95	logistic regression models Mann-Whitney U test Fisher exact test area under the receiver operator characteristics (ROC) curve (AUC) 95% CI	93%	86% NPV: 99%	97	103	Madrid, Spain	40.416775	-3.70379	van Kessel et al., 2017	
85	hematuria	Hematuria	urine								QIAmp DNA Mini-Kit	FGFR3, TERT and HRAS mutation OTX1, ONECUT 2 and TWIST1 methylation	mutation methylation	bisulfite treatment EZ DNA Methylation-Gold QuantiTect Multiplex PCR Master Mix	AUC 0.96 optimism corrected AUC 0.95	logistic regression models Mann-Whitney U test Fisher exact test area under the receiver operator	93%	86% NPV: 99%	97	103	Uppsala, Sweden	59.853642	17.634966	van Kessel et al., 2017	

																characteristics (ROC) curve (AUC) 95% CI									
86	colorectal cancer	Colorectal Cancer	feces					2		2-5 µL bisulfite-converted DNA	methylation: QIAamp DNA Stool Mini Kit expression: RNeasy Mini Kit	GRIA4 and VIPR2 GRIA4, VIPR2, SPOCK1 and SLC6A3 mRNA and proteins	methylation expression	bisulfite conversion EZ DNA Methylation Gold Kit TM MethyLight qPCR TaqMan Genotyping Master mix positive control: Human Methylated & Non-Methylated (WGA) DNA Set reference gene: Alu digital droplet PCR ddPCR Supermix expression: mRNA: SsoAdvanced Universal SYBR® Green Supermix protein: Western blot		Welch's t-test	90%		10		Cagliari, Italy	39.227779	9.111111	Vega-Benedetti et al., 2020	
87	prostate cancer	Prostate Cancer	urine	20 – 120 mL	50 uL			5		5 uL modifed DNA	centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 1: 55%	cohort 1: 80%	cohort 1: 121 cohort 2: 113		Little Rock, Arkansas	34.746483	-92.289597	Vener et al., 2008	
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification:	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC	cohort 1: 55%	cohort 1: 80%	cohort 1: 121 cohort 2: 113		Rochester, Minnesota	44.016369	-92.475395	Vener et al., 2008	

											Picogreen DNAQuantification Assay			polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA		95% CI										
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 1: 55%	cohort 1: 80%	cohort 1: 121 cohort 2: 113		San Diego, California	32.715736	-117.161087	Vener et al., 2008	
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 1: 55%	cohort 1: 80%	cohort 1: 121 cohort 2: 113		Tucson, Arizona	32.25346	-110.911789	Vener et al., 2008	
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 1: 55%	cohort 1: 80%	cohort 1: 121 cohort 2: 113		Snellville, Georgia	33.857327	-84.019913	Vener et al., 2008	

														internal control: ACTB negative control: Universally unmethylated DNA											
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 1: 55%	cohort 1: 80%	cohort 1: 121 cohort 2: 113		Innsbruck, Austria	47.259659	11.400375	Vener et al., 2008	
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 1: 55%	cohort 1: 80%	cohort 1: 121 cohort 2: 113		Graz, Austria	47.076668	15.421371	Vener et al., 2008	
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 1: 55%	cohort 1: 80%	cohort 1: 121 cohort 2: 113		Warsaw, Poland	52.237049	21.017532	Vener et al., 2008	

														unmethylated DNA											
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 2: 53%	cohort 2: 76%	cohort 1: 121 cohort 2: 113		Little Rock, Arkansas	34.746483	-92.289597	Vener et al., 2008	
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 2: 53%	cohort 2: 76%	cohort 1: 121 cohort 2: 113		Rochester, Minnesota	44.016369	-92.475395	Vener et al., 2008	
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 2: 53%	cohort 2: 76%	cohort 1: 121 cohort 2: 113		San Diego, California	32.715736	-117.161087	Vener et al., 2008	
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model	cohort 2: 53%	cohort 2: 76%	cohort 1: 121 cohort 2: 113		Tucson, Arizona	32.25346	-110.911789	Vener et al., 2008	

											Puregene Kit DNA quantification: Picogreen DNAQuantification Assay			quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA		AUC of ROC 95% CI									
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 2: 53%	cohort 2: 76%	cohort 1: 121 cohort 2: 113		Snellville, Georgia	33.857327	-84.019913	Vener et al., 2008	
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 2: 53%	cohort 2: 76%	cohort 1: 121 cohort 2: 113		Innsbruck, Austria	47.259659	11.400375	Vener et al., 2008	
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP)	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 2: 53%	cohort 2: 76%	cohort 1: 121 cohort 2: 113		Graz, Austria	47.076668	15.421371	Vener et al., 2008	

														Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA										
87	prostate cancer	Prostate Cancer	urine								centrifuge, pellet in PBS DNA extraction with Puregene Kit DNA quantification: Picogreen DNAQuantification Assay	GSTP1, RARB, and APC	methylation	EZ-DNA Methylation Kit quantitative methylation-specific polymerase chain reaction (MSP) Taq (Ab) Polymerase internal control: ACTB negative control: Universally unmethylated DNA	cohort 1: AUC 0.69 cohort 2: AUC 0.65	logistic regression model AUC of ROC 95% CI	cohort 2: 53%	cohort 2: 76%	cohort 1: 121 cohort 2: 113		Warsaw, Poland	52.237049	21.017532	Vener et al., 2008	
88	non-muscle-invasive urothelial carcinoma	Bladder Cancer	urine	25-50 mL			0.5		0.0133	500 ng of bisulfite-converted DNA	QIAamp DNA Mini Kit concentration analysis: NanoDrop	BCL2 and hTERT promoters	methylation	bisulfite treatment Epitect Bisulfite Kit positive control: human sperm DNA methylated using SssI DNA methylase, bisulfite treated negative control: untreated sperm DNA methylation-specific real-time polymerase chain reaction QuantiTect Probe polymerase chain reaction Master Mix reference gene: β-Actin	bladder tumor and urine sediment correlation BCL2 and hTERT: P < 0.001 BCL2 only: P = 0.005	Kolmogorov-Smirnov test chi-square test Student's t-test Spearman's rank correlation test receiver operating characteristic curves, area under the curve (AUC) P < 0.05	BCL2 and hTERT: 76%	BCL2 and hTERT: 98%	108	105	Pisa, Italy Florence, Italy	43.716667	10.4	Vinci et al., 2011	BCL2, hTERT, and DAPK promoters
89	colorectal cancer	Colorectal Cancer	feces	250 mg	50 uL			3		3 uL bisulfite-	centrifuged, pellet washed in PBS	SFRP2 promoter	methylation	EpiTect® Bisulfite Kit	hypermethylated: CRCs:	two sided Student's t test	CRCs: 87.0%		69 CRC patients	30	Yangzhou, Jiangsu	32.377529	119.398018	Wang & Tang, 2008	

										converted DNA	QIAamp DNA Stool Mini Kit			Fluorescence-based real-time PCR assay (MethyLight) SYBR greenland PCR standard universal methylated DNA	91.3% advanced adenomas : 79.4% hyperplastic polyps: 53.8%	Chi-square test Fisher exact test P < 0.05					Province, China				
89	colorectal cancer	Colorectal Cancer	feces								centrifuged, pellet washed in PBS QIAamp DNA Stool Mini Kit	SFRP2 promoter	methylation	EpiTect® Bisulfite Kit Fluorescence-based real-time PCR assay (MethyLight) SYBR greenland PCR standard universal methylated DNA	hypermethylated: CRCs: 91.3% advanced adenomas : 79.4% hyperplastic polyps: 53.8%	two sided Student's t test Chi-square test Fisher exact test P < 0.05	advanced adenomas : 61.8%		34 patients with adenoma ≥ 1 cm	30	Yangzhou, Jiangsu Province, China	32.377529	119.398018	Wang & Tang, 2008	
89	colorectal cancer	Colorectal Cancer	feces								centrifuged, pellet washed in PBS QIAamp DNA Stool Mini Kit	SFRP2 promoter	methylation	EpiTect® Bisulfite Kit Fluorescence-based real-time PCR assay (MethyLight) SYBR greenland PCR standard universal methylated DNA	hypermethylated: CRCs: 91.3% advanced adenomas : 79.4% hyperplastic polyps: 53.8%	two sided Student's t test Chi-square test Fisher exact test P < 0.05	hyperplastic polyps: 42.3%	hyperplastic polyps: 76.8%	26 patients with hyperplastic polyp	30	Yangzhou, Jiangsu Province, China	32.377529	119.398018	Wang & Tang, 2008	
90	systemic lupus erythematosus (SLE)	Systemic Lupus Erythematosus (SLE)	urine								centrifuged, pellet analyzed RNA: mirVana PARIS Kit	miR-200a, miR-200c, miR-141, miR-429 and miR-192	microRNA expression	RT-qPCR TaqMan miRNA reverse transcription kit	patients: miR-200a:18.33, miR-200c: 18.36 miR-141: 16.24 miR-429: 14.07 miR-192: 16.04 controls: miR-200a: 20.08 miR-200c: 20.45 miR-141: 17.69 miR-429:	Mann–Whitney U test Spearman's rank correlation p < 0.05				40	30	Hong Kong, China	22.302711	114.177216	Wang et al., 2011	

															16.37 miR-192: 17.74										
91	systemic lupus erythematosus (SLE)	Systemic Lupus Erythematosus (SLE)	urine								centrifuged, pellet analyzed RNA: MirVana™ miRNA isolation kits	miR-146a and miR-155	microRNA expression	RT-qPCR TaqMan miRNA reverse transcription kit	miR-146a & glomerular filtration rate (r = 0.242, P = 0.008) miR-155 & proteinuria (r = 0.407, P < 0.001) & systemic lupus erythematosus disease activity index (r = 0.278, P = 0.002) miR-146a reversely correlated with the urinary expression of TNF-α (r = −0.247, P = 0.012)	Mann–Whitney U test Spearman's rank correlation p < 0.05			40	13	Hong Kong, China	22.302711	114.177216	Wang et al., 2012	
92	non–muscle-invasive bladder cancer (NMIBC)	Bladder Cancer	urine	10 mL							centrifuge, pellet	15 proprietary DNA methylation biomarkers	methylation	real-time polymerase chain reaction Bladder EpiCheckTM Methylation Test	with low-grade lesions: AUC 0.82 without low-grade lesions: AUC 0.94	AUC of ROC 95% CI	68.2% PPV: 44.8% excluding low-grade: 91.7%	88.0% NPV: 95.1% excluding low-grade: NPV: 99.3%	353		Nijmegen, The Netherlands	51.8425	5.85278	Witjes et al., 2018	
92	non–muscle-invasive bladder cancer (NMIBC)	Bladder Cancer	urine								centrifuge, pellet	15 proprietary DNA methylation biomarkers	methylation	real-time polymerase chain reaction Bladder EpiCheckTM Methylation Test	with low-grade lesions: AUC 0.82 without low-grade lesions: AUC 0.94	AUC of ROC 95% CI	68.2% PPV: 44.8% excluding low-grade: 91.7%	88.0% NPV: 95.1% excluding low-grade: NPV: 99.3%	353		Barcelona, Spain	41.390205	2.154007	Witjes et al., 2018	
92	non–muscle-invasive bladder cancer (NMIBC)	Bladder Cancer	urine								centrifuge, pellet	15 proprietary DNA methylation biomarkers	methylation	real-time polymerase chain reaction Bladder EpiCheckTM Methylation Test	with low-grade lesions: AUC 0.82 without low-grade lesions: AUC 0.94	AUC of ROC 95% CI	68.2% PPV: 44.8% excluding low-grade: 91.7%	88.0% NPV: 95.1% excluding low-grade: NPV: 99.3%	353		Hengelo, The Netherlands	52.26583	6.79306	Witjes et al., 2018	
92	non–muscle-invasive bladder	Bladder Cancer	urine								centrifuge, pellet	15 proprietary DNA methylation	methylation	real-time polymerase chain reaction Bladder EpiCheckTM	with low-grade lesions: AUC 0.82 without	AUC of ROC 95% CI	68.2% PPV: 44.8% excluding	88.0% NPV: 95.1% excluding	353		Würzburg, Germany	49.783333	9.933333	Witjes et al., 2018	

	cancer (NMIBC)											biomarkers		Methylation Test	low-grade lesions: AUC 0.94		low-grade: 91.7%	low-grade: NPV: 99.3%						
92	non–muscle-invasive bladder cancer (NMIBC)	Bladder Cancer	urine								centrifuge, pellet	15 proprietary DNA methylation biomarkers	methylation	real-time polymerase chain reaction Bladder EpiCheckTM Methylation Test	with low-grade lesions: AUC 0.82 without low-grade lesions: AUC 0.94	AUC of ROC 95% CI	68.2% PPV: 44.8% excluding low-grade: 91.7%	88.0% NPV: 95.1% excluding low-grade: NPV: 99.3%	353		Kfar Saba, Israel	32.175	34.90694	Witjes et al., 2018	
92	non–muscle-invasive bladder cancer (NMIBC)	Bladder Cancer	urine								centrifuge, pellet	15 proprietary DNA methylation biomarkers	methylation	real-time polymerase chain reaction Bladder EpiCheckTM Methylation Test	with low-grade lesions: AUC 0.82 without low-grade lesions: AUC 0.94	AUC of ROC 95% CI	68.2% PPV: 44.8% excluding low-grade: 91.7%	88.0% NPV: 95.1% excluding low-grade: NPV: 99.3%	353		Sindelfingen, Germany	48.7	9.01667	Witjes et al., 2018	
93	hematuria	Hematuria	urine	30 mL			0.5		0.0167	500 ng of bisulfite-converted DNA	centrifuged, pellet washed in phosphate buffered saline QIAamp DNA Micro Kit concentration analysis: NanoDrop	HOXA9, PCDH17, POU4F2, and ONECUT2	methylation	bisulfite treatment EZ DNA Methylation-Gold Kit methylation-specific high-resolution melting-curve (MS-HRM) polymerase chain reaction (PCR) ZymoTaq qPCR Premix	AUC 0.871	Mann-WhitneyUtest chi-square test AUC of ROC 95% CI	90.5% PPV: 100%	73.2% NPV: 98%	97	95	Shanghai, China	31.224361	121.46917	Wu et al., 2020	
94	renal cell carcinoma (RCC)	Kidney Cancer	urine	20-50 mL				1		1 µl of bisulfite-converted DNA	centrifuged, pellet washed in phosphate-buffered saline AllPrep DNA Mini kit concentration analysis: NanoDrop	TCF21	methylation	bisulfite treatment EZ-96 DNA Methylation-Gold™ Quantitative pyrosequencing methylation analysis HotStarTaq Master Mix kit		Mann-Whitney U test Spearman's correlation AUC of ROC P < 0.05	79%	100%	33	15	Quanzhou, China	24.91389	118.58583	Xin et al., 2016	
95	colorectal cancer	Colorectal Cancer	feces								homogenization stool DNA extraction kit	SEPT9	methylation	bisulfite conversion kit methylated SEPT9 test duplex methylated qPCR assay PCR mastermix internal control: ACBT		p < 0.05 t test Receiver operating characteristic (ROC) curves	83.30%	92.10%	100	60 paired stool and plasma samples	Xuzhou, China	34.205769	117.284126	Y. Liu et al., 2020	

96	bladder cancer	Bladder Cancer	urine				0.2			200 ng DNA	centrifuge, pellet analyzed TIANamp Micro DNA Kit DP316 concentration analysis: Qubit™ 2.0 fluorometer 1% agarose gel > 200 ng, size > 5 Kb	PCDH17 and POU4F2	methylation	bisulfite modification EZ DNA Methylation-Gold™ Kit positive control: CpGenome™ Universal Methylated DNA qMSP SYBR® Premix Ex TaqTM II reference gene: ALU-C4		Mann-Whitney test AUC or ROC 95% CI	90.00%	94%	72 urothelial cell carcinoma 21 infected urinary calculi 26 kidney cancer 22 prostate cancer	23 healthy individuals	He Fei Shi, An Hui Sheng, China	31.871727	117.330551	Y. Wang et al., 2016	
96	bladder cancer	Bladder Cancer	urine								centrifuge, pellet analyzed TIANamp Micro DNA Kit DP316 concentration analysis: Qubit™ 2.0 fluorometer 1% agarose gel > 200 ng, size > 5 Kb	PCDH17 and POU4F2	methylation	bisulfite modification EZ DNA Methylation-Gold™ Kit positive control: CpGenome™ Universal Methylated DNA qMSP SYBR® Premix Ex TaqTM II reference gene: ALU-C4		Mann-Whitney test AUC or ROC 95% CI	90.00%	94%	72 urothelial cell carcinoma 21 infected urinary calculi 26 kidney cancer 22 prostate cancer	23 healthy individuals	Guangzhou, China	23.128994	113.25325	Y. Wang et al., 2016	
96	bladder cancer	Bladder Cancer	urine								centrifuge, pellet analyzed TIANamp Micro DNA Kit DP316 concentration analysis: Qubit™ 2.0 fluorometer 1% agarose gel > 200 ng, size > 5 Kb	PCDH17 and POU4F2	methylation	bisulfite modification EZ DNA Methylation-Gold™ Kit positive control: CpGenome™ Universal Methylated DNA qMSP SYBR® Premix Ex TaqTM II reference gene: ALU-C4		Mann-Whitney test AUC or ROC 95% CI	90.00%	94%	72 urothelial cell carcinoma 21 infected urinary calculi 26 kidney cancer 22 prostate cancer	23 healthy individuals	Guang Zhou Shi, Guang Dong Sheng, China	23.128994	113.25325	Y. Wang et al., 2016	
97	urothelial cancer	Bladder Cancer	urine	100 mL			1		0.0100	1 ug bisulfite-treated DNA	centrifuged, pellet washed in phosphate-buffered saline	RASSF1a, APC and E-cad promoters	methylation	quantitative fluorometric real-time methylation-specific PCR assay (QMSP) normalization gene: β-Actin		t test chi-square test	RASSF1a: 0.51 APC: 0.4 E-cad: 0.31 RASSF1a, APC and E-cad panel: 0.69	RASSF1a: 0.63 APC: 0.83 E-cad: 0.53 RASSF1a, APC and E-cad	35	35 over 70 years old 34 under 40 years old	Sheffield, United Kingdom	53.383331	-1.466667	Yates et al., 2006	All biomarkers: RASSF1a, E-Cadherin, APC, p16, p14, GSTP, DAPK and RARb promoters

																		panel: 0.6							
98	bladder cancer	Bladder Cancer	urine				0.2			~200 ng bisulfite-modified DNA	ZR Urine DNA Isolation Kit	TWIST1 and NID2	methylation	bisulfite treatment EZ DNA Methylation-Gold Kit methylation-specific polymerase chain reaction (MSP) ZymoTaq DNA Polymerase positive control: Universal methylated human DNA standard		Fisher's exact tests P < 0.05	87.50%	95.80%	24	15	Samsun, Turkey	41.27976	36.3361	Yegin et al., 2013	
99	urothelial carcinoma	Bladder Cancer	urine	50 mL			1		0.0200	1 µg of total RNA	centrifuged, pellet washed in PBS Genomic DNA Mini Kits	ZNF671, IRF8 and sFRP1	methylation	bisulfite treatment: EZ DNA methylation kits Quantitative MSP reference gene: β-Actin		Mann-Whitney tests Kaplan-Meier analysis Cox hazard-proportional regression analysis AUC of ROC 95% CI	42%-48% ZNF671, IRF8 and sFRP1: 96.2%	89%-92.8%	61	28	Chia-Yi, Taiwan	47.4925	19.0513	Yeh et al., 2015	
100	bladder cancer	Bladder Cancer	urine								proteinase K/organic extraction method	SALL3, CFTR, ABCC6, HPR1, RASSF1A, MT1A, RUNX3, ITGA4, BCL2, ALX4, MYOD1, DRM, CDH13, BMP3B, CCNA1, RPRM, MINT1, and BRCA1	methylation	bisulfite treatment MSP		Fisher's exact test AUC of ROC 95% CI	91.70%	87%	132	23	Nanning, Guangxi, China	22.816668	108.316666	Yu et al., 2007	
100	bladder cancer	Bladder Cancer	urine								proteinase K/organic extraction method	SALL3, CFTR, ABCC6, HPR1, RASSF1A, MT1A, RUNX3, ITGA4, BCL2,	methylation	bisulfite treatment MSP		Fisher's exact test AUC of ROC 95% CI	91.70%	87%	132	23	Shanghai, China	31.224361	121.46917	Yu et al., 2007	

												ALX4, MYOD1, DRM, CDH13, BMP3B, CCNA1, RPRM, MINT1, and BRCA1													
101	prostate cancer	Prostate Cancer	urine					1		1 uL bisulfite-treated DNA	centrifuge, pellet in PBS	DLEC1	methylation	bisulfite conversion EZ DNA Methylation-Direct Kit MSP		t test Fisher exact test chi-square test p <0.05	PrCa patients: 36.7%		30 prostate cancer (PrCa)	30 benign prostatic hyperplasia (BPH)	Beijing, China	39.916668	116.383331	Zhang et al., 2015	
102	hematuria	Hematuria	urine		30 uL			1		1 uL DNA	QIAamp DNA Micro Kit concentration analysis: NanoDrop	HOXA9, ONECUT2, PCDH17, PENK, TWIST1, VIM and ZNF154	methylation	bisulfite treatment EZ DNA Methylation-Gold™ Kit ZymoTaq™ qPCR Premix	AUC 0.894 and 0.851	Pearson's Chi-square test Fisher's exact test logistic regression model AUC of ROC 95% CI			99		Shanghai, China	31.224361	121.46917	Zhang et al., 2019	
103	prostate cancer	Prostate Cancer	urine	20–80 mL			0.005		0.0001	5 ng/μl DNA	centrifuged, pellet suspended in phosphate buffered saline QiaAMP DNA Micro Kit concentration analysis: NanoDrop	APC, CRIP3, GSTP1, HOXD3, HOXD8, KLK10, TBX15 and TGFb2	methylation	bisulfite treatment EZ DNA Methylation-Gold Kit multiplex MethyLight normalization control: ALU	Classifier: 2.695 OR and Clinical: 2.734	Spearman p test Mann-Whitney U test logistic regression 95% CI			153		Toronto, Ontario, Canada	43.65107	-79.347015	Zhao et al., 2017	Online options are not free, sent a Report a Problem request for ILL
104	prostate cancer	Prostate Cancer	urine	50 mL	1 mL						centrifuge, pelle in PBS AllPrep DNA/RNA mini-kit	HOXD3 and GSTP1	methylation	bisulfite conversion quantitative MethyLight assay Prostate Cancer Urinary Epigenetic (ProCUrE) assay positive control: CpGenome Universal Methylated DNA reference gene: ALU		Spearman's ρ rank chi-square tests logistic regression ROC 95% CI C-statistic	31.6% PPV: 59.4–78%	false positive: 11.9%	408		Norwich, Norfolk, England	52.630886	1.297355	Zhao et al., 2018	
104	prostate cancer	Prostate Cancer	urine								centrifuge, pelle in PBS	HOXD3 and GSTP1	methylation	bisulfite conversion		Spearman's ρ rank chi-square	31.6% PPV: 59.4–78%	false positive: 11.9%	408		Toronto, Ontario, Canada	43.65107	-79.347015	Zhao et al., 2018	

											AllPrep DNA/RNA mini-kit			quantitative MethyLight assay Prostate Cancer Urinary Epigenetic (ProCUrE) assay positive control: CpGenome Universal Methylated DNA reference gene: ALU		tests logistic regression ROC 95% CI C-statistic									
104	prostate cancer	Prostate Cancer	urine								centrifuge, pelle in PBS AllPrep DNA/RNA mini-kit	HOXD3 and GSTP1	methylation	bisulfite conversion quantitative MethyLight assay Prostate Cancer Urinary Epigenetic (ProCUrE) assay positive control: CpGenome Universal Methylated DNA reference gene: ALU		Spearman's ρ rank chi-square tests logistic regression ROC 95% CI C-statistic	31.6% PPV: 59.4–78%	false positive: 11.9%	408		Dublin, Ireland	53.35014	-6.266155	Zhao et al., 2018	
105	prostate cancer	Prostate Cancer	urine	20–80 mL	45 mL		0.1		0.0020	100 ng of bisulfite-converted DNA	centrifuge, pellet washed in PBS RNA: miRNeasy Serum/Plasma kit DNA: QiaAMP DNA micro kit concentration analysis: NanoDrop	miR-24, miR-30c and CRIP3	expression methylation	miRNA expression: miRCURY LNA miRNA SYBR Green PCR system DNA methylation: EZ DNA Methylation-Gold kit qPCR-based MethyLight assay normalization control: ALU-C4 (ALU) repeats	Odds ratio = 2.166 patient reclassification: AUC of 0.708	Spearman's r ranked test Backward step-wise logistic regression Mann-Whitney U AUC of ROC 95% CI	81%	59.7% NPV: 90.9%	103		Toronto, Ontario, Canada	43.65107	-79.347015	Zhao et al., 2019	
106	bladder cancer	Bladder Cancer	urine								Wizard Plus Minipreps DNA Purificiation System (Promega) guanidine thiocyanate, resin mixing	jagged edges	methylation	bisulfite conversion EpiTect Plus Bisulfite Kit Illumina sequencing	JI-U values median: 35.8; range: 21.3–60.0 AUC: 0.75	Mann-Whitney U test JI-U 95% CI			46	39	Hong Kong, China	22.302711	114.177216	Zhou et al., 2021	

107	colorectal cancer	Colorectal Cancer	feces	2 g			2			2.0 µg of bisulfite-converted DNA	centrifuged, pellet in GT buffer 1 proteinase K concentration analysis: Qubit dsDNA BR assay kit	SDC2	methylation	bisulfite treatment EZ DNA Methylation-Gold kit linear target enrichment (LTE)-quantitative methylation-specific real-time PCR (qMSP) AptaTaq PCR master mix	AUC of 0.902	AUC of ROC 95% CI	90.2% early stages: 89.1%	90.20%	62	245	Seoul, South Korea	37.5326	127.024612	Han et al., 2019	
108	colorectal cancer	Colorectal Cancer	feces				2			2 µg DNA	e QiaAmp DNA Stool mini-kit	SFRP2, GATA4/5, NDRG4 and VIM	methylation	bisulfite treatment EpiTect Bisulfite Kit Methylation-specific polymerase chain reaction 2.5% agarose gel, UV	Promoter methylation levels SFRP2: 57.1% GATA4/5: 42.9%, 83.9% NDRG4: 28.6% VIM: 41.1%	chi-square test Fisher's exact tests AUC of ROC 95% CI	at least one methylated gene: 96.4%	at least one methylated gene: 65%	56	40	Ningbo, China	29.868336	121.543991	Lu et al., 2014	
109	colorectal cancer	Colorectal Cancer	feces					2		2 µL DNA	Stool DNA Extraction kit	NDRG4	methylation	bisulfite treatment EZ DNA Methylation TM-Direct kit n-MSP Taq Polymerase			positive detection: feces: 76.2%		76	16	Beijing, China	39.916668	116.383331	Xiao et al., 2015	
109	colorectal cancer	Colorectal Cancer	urine								Stool DNA Extraction kit	NDRG4	methylation	bisulfite treatment EZ DNA Methylation TM-Direct kit n-MSP Taq Polymerase			positive detection: urine: 72.6%		76	16	Beijing, China	39.916668	116.383331	Xiao et al., 2015	