May 4, 2023

Nucleix Ltd.
% Janice Hogan
Partner
Hogan Lovells US LLP
1735 Market Street, Floor 23
Philadelphia, Pennsylvania 19103

Re: K203245
Trade/Device Name: Bladder EpiCheck Kit
Regulation Number: 21 CFR 866.6010
Regulation Name: Tumor-Associated Antigen Immunological Test System
Regulatory Class: Class II
Product Code: MMW
Dated: November 3, 2020
Received: November 3, 2020

Dear Janice Hogan:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal

statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801 and Part 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for devices or postmarketing safety reporting (21 CFR 4, Subpart B) for combination products (see https://www.fda.gov/combination-products/guidance-regulatory-information/postmarketing-safety-reporting-combination-products); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820) for devices or current good manufacturing practices (21 CFR 4, Subpart A) for combination products; and, if applicable, the electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to https://www.fda.gov/medical-devices/medical-device-safety/medical-device-reporting-mdr-how-report-medical-device-problems.

For comprehensive regulatory information about medical devices and radiation-emitting products, including information about labeling regulations, please see Device Advice (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance) and CDRH Learn (https://www.fda.gov/training-and-continuing-education/cdrh-learn). Additionally, you may contact the Division of Industry and Consumer Education (DICE) to ask a question about a specific regulatory topic. See the DICE website (https://www.fda.gov/medical-devices/device-advice-comprehensive-regulatory-assistance/contact-us-division-industry-and-consumer-education-dice) for more information or contact DICE by email (DICE@fda.hhs.gov) or phone (1-800-638-2041 or 301-796-7100).

Sincerely,

Zivana Tezak-fragale -S

Zivana Tezak
Branch Chief
Division of Molecular Genetics
and Pathology
OHT7: Office of In Vitro Diagnostics
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES Food and Drug Administration **Indications for Use**	Form Approved: OMB No. 0910-0120 Expiration Date: 06/30/2023 *See PRA Statement below*

510(k) Number *(if known)*

K203245

Device Name

Bladder EpiCheck Kit

Indications for Use *(Describe)*

Bladder EpiCheck Kit is intended for the qualitative detection of DNA methylation patterns of 15 loci in human DNA that are associated with transitional cell carcinoma of the bladder. The test is performed on voided urine samples and run on the ABI® 7500 Fast Dx Real-Time PCR system.

Bladder EpiCheck Kit is indicated for use as a non-invasive method to monitor for tumor recurrence in conjunction with cystoscopy in patients previously diagnosed with Non-Muscle Invasive Bladder Cancer.

Type of Use *(Select one or both, as applicable)*

X Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

CONTINUE ON A SEPARATE PAGE IF NEEDED.

This section applies only to requirements of the Paperwork Reduction Act of 1995.

DO NOT SEND YOUR COMPLETED FORM TO THE PRA STAFF EMAIL ADDRESS BELOW.

The burden time for this collection of information is estimated to average 79 hours per response, including the time to review instructions, search existing data sources, gather and maintain the data needed and complete and review the collection of information. Send comments regarding this burden estimate or any other aspect of this information collection, including suggestions for reducing this burden, to:

Department of Health and Human Services
Food and Drug Administration
Office of Chief Information Officer
Paperwork Reduction Act (PRA) Staff
PRAStaff@fda.hhs.gov

"An agency may not conduct or sponsor, and a person is not required to respond to, a collection of information unless it displays a currently valid OMB number.

510(k) Summary
for Bladder EpiCheck Test

1. Submitter

Nucleix, Ltd.
Pekeris 3
Rehovot 7670203, Israel

Contact: Eli Frydman, Ph.D.
President, EMEA
Email: eli@nucleix.com

2. Submission Correspondent - US

Janice Hogan
Hogan Lovells US LLP
1735 Market Street, Suite 2320
Philadelphia, PA 19103
Janice.hogan@hoganlovells.com

3. Device Identification

Name of Device:	Bladder EpiCheck® Test
Common or Usual Name:	Bladder EpiCheck
Classification Name	Tumor-associated antigen immunological test system
Classification Regulation:	866.6010
Product Code:	MMW
Device Class:	Class II
Classification Panel:	Immunology

4. Legally Marketed Predicate Device

Abbott Molecular UroVysion™ Bladder Cancer Kit (K013785)

5. Device Description

The Bladder EpiCheck Test is a real-time PCR-based *in vitro* diagnostic assay intended for the qualitative detection of DNA methylation patterns associated with transitional cell carcinoma of the bladder to monitor for tumor recurrence (in conjunction with cystoscopy) in patients previously diagnosed with non-muscle invasive bladder cancer (NMIBC).

The assay consists of a panel of 15 novel DNA methylation (covalent addition of methyl (CH3) groups to the C5 position of the pyrimidine ring of cytosines, typically in a CpG dinucleotide) biomarkers that were found to distinguish between patients with bladder cancer and patients without bladder cancer. The Bladder EpiCheck Test differentiates between methylated and non-methylated DNA, creating a unique platform for methylation profiling of urine specimens towards the detection of bladder cancer recurrence in patients previously diagnosed with the disease. The test is comprised of reagents for end-to-end (sample-to-answer) processing of urine samples (reagents for DNA extraction, DNA digestion, PCR amplification, and analysis software), and is performed using the Applied Biosystems® 7500 Fast Dx Real-Time PCR system.

A voided urine specimen is centrifuged, and the cells (both normal and cancerous if present) are separated from the urine supernatant. DNA is then extracted from the cell pellet using the Bladder EpiCheck Extraction kit (P/N NX899090-01C). The extracted DNA is digested using a methylation-sensitive restriction enzyme mix, which cleaves DNA at specific recognition sequences if they are unmethylated. Methylated DNA is protected from enzymatic digestion and therefore remains intact.

Real-Time PCR Amplification
Using reagents provided in the Bladder EpiCheck Test kit, the digested DNA samples and test controls are mixed together with PCR primers and probe into 8 unique PCR multiplexes reactions and assayed using the ABI 7500 Fast Dx Real-Time PCR system. The panel of 15 biomarkers is amplified according to their residual levels of methylation. Real-time PCR amplification is performed in a 96-well plate, requires 2 assay-control samples (Undigested Control and No Template Control) and can test up to 10 patient samples. Each sample is analyzed in 8 wells, corresponding to a single column of the plate. In each well, 3-4 biomarkers are amplified. The panel of 15 biomarkers are used to determine whether a sample is bladder cancer positive or bladder cancer negative. In addition, 3 internal controls (Internal Reference, Digestion Control, and Digestion Control Reference) are used to verify successful digestion and amplification processes.

Data Analysis
The Bladder EpiCheck software (P/N NX899090-03C) accepts as an input (Source File) the *.SDS output file of the ABI7500 DX real-time PCR machine, and outputs for each patient's sample a qualitative diagnosis (positive/negative for recurrence of bladder cancer) based on the patient's EpiScore. The software implements the Bladder EpiCheck algorithm, which starts from the raw fluorescence data of the real-time PCR run and ends in the diagnosis (positive/negative) of each patient. The software enables the operator,

who is running the test, to view and export reports of each patient, as well as to view the raw data of the real-time PCR run. The Bladder EpiCheck software is installed on a stand-alone PC; it does not require web connection. The software setup installs 3rd-party specific templates, which contain all required data and steps for the PCR run on the ABI 7500 Dx Real-time PCR machine. The user only needs to input the sample's IDs in the third-party software and upload the output file once the PCR run is completed. The Bladder EpiCheck software then parses the source file, and translates its content to a unified internal format, Nucleix Real-time Information XML (NRIX). Once in NRIX format, the Bladder EpiCheck algorithm processes the signals, while removing background noise and anomalies, and interpolates the distinct cycle data. The algorithm identifies the sample's well location (controls and test) and validates their raw data and each calculation steps against predefined thresholds. Once the algorithm run is completed, the EpiScore and diagnosis are assigned to each sample unless an error occurred at any of the calculation steps. The software's user interface is designed to clearly correlate between the sample's location and their test results. A simple color-coded scheme separates between negative, positive, or invalid results and from clinical samples and kit control samples (UC and NTC). Per sample view is also available to see the sample's fluorescence signals, a sigmoid-like amplification curve for each marker. The patient's qualitative results and the overall run summary report can be viewed in the software's user interface and exported as PDF file.

Result Reporting

The Bladder EpiCheck Test Kit is used to calculate the associated methylation level for each of the 15 biomarkers based on the associated Cq (Cycle of quantitation, which is the PCR cycle at which the marker's amplification curve reached a predefined signal threshold). These methylation levels are translated to marker scores that are based on reference methylation levels obtained from a large set of bladder cancer negative and positive patients. These 15 marker scores are then combined into a single number: the EpiScore, a number between 0 and 100 representing the overall methylation level of the sample at the panel biomarkers. The resulting EpiScore is then compared to a pre-specified threshold; if it is above or equal to the threshold (60), the test is called "Positive"; otherwise, it is called "Negative".

If the sample analysis is failed (e.g., insufficient DNA, incomplete digestion) or the analysis of the entire PCR plate failed (e.g., one of the plate controls failed), the output for the sample will be "Invalid". In some cases of failure, the sample/plate run may be repeated a second time, and in other cases the user will be instructed to collect a new sample from the patient.

6. Intended Use Statement

Bladder EpiCheck Kit is intended for the qualitative detection of DNA methylation patterns of 15 loci in Human DNA that are associated with transitional cell carcinoma of the bladder. The test is performed on voided urine samples and run on the ABI 7500 Fast Dx Real-Time PCR system.

Bladder EpiCheck Kit is indicated for use as a non-invasive method to monitor for tumor recurrence in conjunction with cystoscopy in patients previously diagnosed with non-muscle invasive bladder cancer.

Special conditions for Use statement(s):

- For prescription use only
- For *in vitro* diagnostic use

Special instrument requirements:

- Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument, SDS Software version 1.4 (cleared by FDA, K082562)

7. Comparison of Technological Characteristics with The Predicate Device

Bladder EpiCheck is substantially equivalent to the Abbott Molecular UroVysion Bladder Cancer Kit (K013785). The Bladder EpiCheck test has the same general intended use as the previously cleared predicate device. Although the technological characteristics and principles of operation are different between Bladder EpiCheck and UroVysion, those changes do not raise any new questions of safety and efficacy. Both the Bladder EpiCheck test and UroVysion kit start with the same patient specimen type.

Table 7.1 Similarities between the Predicate and Subject Device

Characteristic	Predicate UroVysion Bladder Cancer Recurrence Kit (K013785)	Subject Device Bladder EpiCheck
Similarities		
Indications for Use	The UroVysion Bladder Cancer Recurrence kit is designed to detect aneuploidy for chromosomes 3, 7, 17 and loss of the 9p21 locus via fluorescence in situ hybridization (FISH) in urine specimens from subjects with transitional cell carcinoma of the bladder. Results from the UroVysion Bladder Cancer Recurrence Kit are intended for use as a noninvasive method for monitoring for tumor recurrence in conjunction with cystoscopy in	Bladder EpiCheck Kit is intended for the qualitative detection of DNA methylation patterns of 15 loci in Human DNA that are associated with transitional cell carcinoma of the bladder. The test is performed on voided urine samples and run on the ABI 7500 Fast Dx Real-Time PCR system. Bladder EpiCheck Kit is indicated for use as a non-invasive method to monitor for tumor recurrence in conjunction with cystoscopy in

	patients previously diagnosed with bladder cancer.	patients previously diagnosed with Non-Muscle Invasive Bladder Cancer.
Measurement Type	Qualitative	Same
Specimen Type	Voided Urine	Same
Sample Collection Method	Non-invasive collection of biological samples delivered into a non-sterile plastic collection cap/tube	Same
Special Conditions for Use	Prescription Only	Same

Table 7.2 Differences between the Predicate and Subject Device

Characteristic	**Predicate UroVysion Bladder Cancer Recurrence Kit (K013785)**	**Subject Device Bladder EpiCheck**
Differences		
Principle of the Procedure	Fluorescence In situ hybridization (FISH)	Real-Time PCR Reaction (RT-PCR)
Test Target	Chromosomal DNA	DNA Methylation Biomarkers
Extraction and Assay Preparation	Cells recovered from urine pellets are fixed on slides. DNA is denatured to its single stranded form and subsequently allowed to hybridize with the probes.	DNA is extracted from the cell pellet recovered from urine. DNA is digested using a methylation-sensitive restriction enzyme, which cleaves DNA at its recognition sequence if it is unmethylated.
Detection Instrument	Epi-fluorescence microscope	FDA-cleared Applied Biosystems® 7500 Fast Dx Real-Time PCR Instrument
Assay Controls	Control slides are run concurrently with patient slides.	Controls are run concurrently with patient samples. Three controls consisting of Internal Reference Control (IR), No Template Control (NTC) and Undigested Control (UC)

8. Performance Testing (Analytical)

8.1 Precision/Reproducibility

Laboratory-to-Laboratory

A laboratory-to-laboratory repeatability and reproducibility study was performed to assess variation of Bladder EpiCheck when performed in 3 different laboratories by 2 operators at each laboratory site over 3 non-consecutive run days using contrived human DNA samples. Six (6) contrived human DNA samples were created by blending gDNA from different sample sources (T24 cell line DNA (T24) and commercially available DNA (Cml.), representing different Bladder EpiCheck marker combinations) to represent four different EpiScore categories (Low Negative (LN), High Negative (HN), Low Positive (LP), and High Positive (HP)), including scores around the test cutoff (high negative and low positive). These 6 samples were tested in technical duplicates at 2 different inputs (Low = 4ng/PCR; High = 10ng/PCR). Overall performance characteristics (Agreement, PPA, and NPA) are shown in Tables 8.1.1, 8.1.2 and 8.1.3 below. There were no control (plate) failures (0/54); overall invalid rate was 0.2% (1/433; 95% CI 0.05%; 1.03%).

Table 8.1.1 Interlaboratory Reproducibility per Sample Presented as Overall and by Day

		Bladder EpiCheck Result									
		Timepoint (Day)						**Overall**			
		1		**2**		**3**					
Negative Samples	**DNA Input**	**NPA**		**NPA**		**NPA**		**NPA**		**Invalid Rate**	
		% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI
T24 LN	High	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (36/36)	[93.01%; 100%]	1.4% (1/73)	[0.31%; 5.91%]
	Low	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (36/36)	[93.01%; 100%]		
T24 HN	High	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (36/36)	[93.01%; 100%]	0	--
	Low	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (36/36)	[93.01%; 100%]		
Cml. HN	High	100% (12/12)	[81.60%; 100%]	91.7% (11/12)	[69.88%; 98.12%]	100% (12/12)	[81.60%; 100%]	97.2% (35/36)	[88.46%; 99.38%]	0	--
	Low	100% (12/	[81.60%; 100%	83.3% (10/	[60.08%; 94.32	100% (12/12)	[81.60%; 100%]	94.4% (34/36)	[84.53%; 98.14		

		12)	]	12)	%]				%]		
All Negatives		100 % (72/ 72)	[96.3 8%; 100%]	95.8 % (69/ 72)	[90.02 %; 95.83 %]	100% (72/7 2)	[96.38 %; 100%]	98.6% (213/2 16)	[96.58 %; 99.44 %]	0.5% (1/21 7)	[0.10 %; 2.04 %]
Positive Samples	**DNA Input**	**PPA**		**PPA**		**PPA**		**PPA**		**Invalid Rate**	
		% (n/ N)	95% CI	% (n/ N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI
T24 LP	High	100 % (12/ 12)	[96.3 8%; 100%]	100 % (12/ 12)	[81.60 %; 100%]	100% (12/1 2)	[81.60 %; 100%]	100% (36/36)	[93.01 %; 100%]	0	--
	Low	100 % (12/ 12)	[96.3 8%; 100%]	100 % (12/ 12)	[81.60 %; 100%]	100% (12/1 2)	[81.60 %; 100%]	100% (36/36)	[93.01 %; 100%]		
T24 HP	High	100 % (12/ 12)	[96.3 8%; 100%]	100 % (12/ 12)	[81.60 %; 100%]	100% (12/1 2)	[81.60 %; 100%]	100% (36/36)	[93.01 %; 100%]	0	--
	Low	100 % (12/ 12)	[96.38 %; 100%]	100 % (12/ 12)	[81.60 %; 100%]	100% (12/12)	[81.60 %; 100%]	100% (36/36)	[93.01 %; 100%]		
Cml. LP	High	100 % (12/ 12)	[96.38 %; 100%]	100 % (12/ 12)	[81.60 %; 100%]	100% (12/12)	[81.60 %; 100%]	100% (36/36)	[93.01 %; 100%]	0	--
	Low	100 % (12/ 12)	[96.38 %; 100%]	100 % (12/ 12)	[81.60 %; 100%]	100% (12/12)	[81.60 %; 100%]	100% (36/36)	[93.01 %; 100%]		
All Positives		100 % (72/ 72)	[96.38 %; 100%]	100 % (72/ 72)	[96.38 %; 100%]	100% (72/72)	[96.38 %; 100%]	100% (216/2 16)	[98.76 %; 100%]	0	--
All Samples		**Agreement**		**Agreement**		**Agreement**		**Agreement**		**Invalid Rate**	
		% (n/ N)	95% CI	% (n/ N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI
Overall		100 % (144/ 144)	[98.16 %; 100%]	97.9 % (141 /144)	[97.92 %; 99.16 %]	100% (144/1 44)	[98.16 %; 100%]	99.3% (429/4 32)	[98.28 % 99.72 %]	0.2% (1/43 3)	[0.05 %; 1.03 %]

Table 8.1.2 Interlaboratory Reproducibility per Sample Presented as Overall and by Operator

	Bladder EpiCheck Result												
	Operator												**Overall**
	1		**2**		**3**		**4**		**5**		**6**		
Negative Samples/ Input	**NPA**		**NPA**		**NPA**		**NPA**		**NPA**		**NPA**		**NPA**	
	% (n/ N)	95% CI	% (n/ N)	95% CI	% (n/ N)	95% CI	% (n/ N)	95% CI	% (n/ N)	95% CI	% (n/ N)	95% CI	% (n/N)	95% CI

T24 LN High	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100% (36/3 6)	[93.0 %; 100 %]
T24 LN Low	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100% (36/3 6)	[93.0 %; 100 %]
T24 HN High	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100% (36/3 6)	[93.0 %; 100 %]
T24 HN Low	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100% (36/3 6)	[93.0 %; 100 %]
Cml. HN Low	100 % (6/6)	[68.9 %; 100 %]	83.3 % (5/6)	[49.8 %; 96.19 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68. 9%; 100 %]	97.2 % (35/3 6)	[88.5 %; 99.3 8%]
Cml. HN Low	100 % (6/6)	[68.9 %; 100 %]	66.7 % (4/6)	[34.7 %; 88.27 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68.9 ; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68. 9%; 100 %]	94.4 % (34/3 6)	[84.5 %; 98.1 4%]
All Neg	100 % (36/ 36)	[93.0 %; 100 %]	91.7 % (33/ 36)	[80.9 %; 96.62 %]	100 % (36/ 36)	[93. 0%; 100 %]	100 % (36/ 36)	[93.0 1%; 100 %]	100 % (36/ 36)	[93. 0%; 100 %]	100 % (36/ 36)	[93. 0%; 100 %]	98.6 % (213/ 216)	[96.6 %; 99.4 4%]
Positive Sample/ Input	**PPA**		**PPA**		**PPA**		**PPA**		**PPA**		**PPA**		**PPA**	
	% (n/ N)	95% CI	% (n/ N)	95% CI	% (n/ N)	95% CI	% (n/ N)	95% CI	% (n/ N)	95% CI	% (n/ N)	95% CI	% (n/N)	95% CI
T24 LP High	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100% (36/3 6)	[93.0 %; 100 %]
T24 LP Low	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100% (36/3 6)	[93.0 %; 100 %]
T24 HP High	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68. 9 %; 100 %]	100% (36/3 6)	[93.0 %; 100 %]
T24 HP Low	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68. %; 100 %]	100% (36/3 6)	[93.0 %; 100 %]
Cml. LP High	100 % (6/6)	[68.9 2%; 100 %]	100 % (6/6)	[68.9 2%; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100% (36/3 6)	[93.0 %; 100 %]
Cml. HP Low	100 % (6/6)	[68.9 2%; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68.9 %; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100 % (6/6)	[68. 9%; 100 %]	100% (36/3 6)	[93.0 %; 100 %]
All Pos	100 % (36/ 36)	[93.0 %; 100 %]	100 % (36/ 36)	[93.0 %; 100 %]	100 % (36/ 36)	[93. 0%; 100 %]	100 % (36/ 36)	[93.0 %; 100 %]	100 % (36/ 36)	[93. 0%; 100 %]	100 % (36/ 36)	[93. 0%; 100 %]	100% (216/ 216)	[98.8 %; 100 %]
All Samples	**Agreement**		**Agreement**		**Agreement**		**Agreement**		**Agreement**		**Agreement**		**Agreement**	
	% (n/	95% CI	% (n/	95% CI	% (n/	95% CI	% (n/	95% CI	% (n/	95% CI	% (n/	95% CI	% (n/N)	95% CI

	N)		N)		N)		N)		N)		N)			
Overall	100% (72/72)	[96.4%; 100%]	95.8% (69/72)	[90.0%; 95.83%]	100% (72/72)	[96.4%; 100%]	100% (72/72)	[96.4%; 100%]	100% 72/72	[96.4%; 100%]	100% (72/72)	[96.4%; 100%]	99.3% (429/432)	[98.3% 99.72%]

Table 8.1.3 Interlaboratory Reproducibility per Sample Presented as Overall and by Laboratory

		Bladder EpiCheck Result							
		Laboratory						**Overall**	
		1		**2**		**3**			
Negative Samples	**DNA Input**	**NPA**		**NPA**		**NPA**		**NPA**	
		% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI
T24 LN	High	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (36/36)	[93.01%; 100%]
	Low	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (36/36)	[93.01%; 100%]
T24 HN	High	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (36/36)	[93.01%; 100%]
	Low	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	100% (36/36)	[93.01%; 100%]
Cml. HN	High	91.7% (11/12)	[69.88%; 98.12%]	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	97.2% (35/36)	[88.46%; 99.38%]
	Low	83.3% (10/12)	[60.08%; 94.32%]	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%; 100%]	94.4% (34/36)	[84.53%; 98.14%]
All Negatives		95.8% (69/72)	[90.02%; 95.83%]	100% (72/72)	[96.38%; 100%]	100% (72/72)	[96.38%; 100%]	98.6% (213/216)	[96.58%; 99.44%]
Positive Samples	**DNA Input**	**PPA**		**PPA**		**PPA**		**PPA**	
		% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI
T24 LP	High	100% (12/12)	[81.60%; 100%]	100% (12/12)	[81.60%;	100% (12/12)	[81.60%;	100% (36/36)	[93.01%;

					100 %]		100 %]		100 %]
	Low	100% (12/12)	[81.60 %; 100%]	100% (12/12)	[81. 60% ; 100 %]	100% (12/12)	[81. 60% ; 100 %]	100% (36/36)	[93. 01% ; 100 %]
T24 HP	High	100% (12/12)	[81.60 %; 100%]	100% (12/12)	[81. 60% ; 100 %]	100% (12/12)	[81. 60% ; 100 %]	100% (36/36)	[93. 01% ; 100 %]
	Low	100% (12/12)	[81.60 %; 100%]	100% (12/12)	[81. 60% ; 100 %]	100% (12/12)	[81. 60% ; 100 %]	100% (36/36)	[93. 01% ; 100 %]
Cml. LP	High	100% (12/12)	[81.60 %; 100%]	100% (12/12)	[81. 60% ; 100 %]	100% (12/12)	[81. 60% ; 100 %]	100% (36/36)	[93. 01% ; 100 %]
	Low	100% (12/12)	[81.60 %; 100%]	100% (12/12)	[81. 60% ; 100 %]	100% (12/12)	[81. 60% ; 100 %]	100% (36/36)	[93. 01% ; 100 %]
All Positives		100% (72/72)	[96.38 %; 100%]	100% (72/72)	[96. 38% ; 100 %]	100% (72/72)	[96. 38% ; 100 %]	100% (216/216)	[98. 76% ; 100 %]
All Samples		**Agreement**		**Agreement**		**Agreement**		**Agreement**	
		% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI	% (n/N)	95% CI
Overall		97.9% (141/144)	[97.92 %; 99.16%]	100% (144/144)	[98. 16% ; 100 %]	100% (144/144)	[98. 16% ; 100 %]	99.3% (429/432)	[98. 28% 99.72%]

An additional laboratory-to-laboratory reproducibility study, using clinical samples, was performed at 3 laboratories to determine precision between laboratories. The four (4) clinical samples used represent Low Negative (LN; ES ~20), High Negative (HN; ES~45), Low Positive (HP; ES~75), and High Positive (HP; ES~90) at 2 DNA input levels. One operator ran the test at each laboratory location on 3 different, non-consecutive workdays with an interval of at least 3 days between runs. A single reagent lot was used for this study. Overall performance characteristics (agreement, PPA, and NPA) are described in Tables 8.1.4 and 8.1.5 below. There were no control (plate) failures (0/18).

Table 8.1.4 Interlaboratory Reproducibility per Sample Presented as Overall and by Day

	Bladder EpiCheck Result	
	Laboratory	**Overall**

		1		2		3			
Negative Sample	**DNA Input**	**NPA**		**NPA**		**NPA**		**NPA**	
		% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90% CI
LN	High	100% (6/6)	[78.51 %; 100%]	100% (6/6)	[78.51 %; 100%]	100% (6/6)	[78.51 %; 100%]	100% (18/18)	[91.64 %; 100%]
	Low	100% (6/6)	[78.51 %; 100%]	100% (6/6)	[78.51 %; 100%]	100% (6/6)	[78.51 %; 100%]	100% (18/18)	[91.64 %; 100%]
HN	High	100% (6/6)	[78.51 %; 100%]	83.3% (5/6)	[57.47 %; 94.87 %]	83.3% (5/6)	[57.47 %; 94.87 %]	88.9% (16/18)	[75.99 %; 95.29 %]
	Low	66.7 % (4/6)	[40.94 %; 85.23 %]	100% (6/6)	[78.51 %; 100%]	83.3% (5/6)	[57.47 %; 94.87 %]	83.3% (15/18)	[69.42 %; 91.68 %]
All Negatives		91.7 % (22/24)	[75.1 %; 98.2%]	95.8% (23/24)	[80.4 %; 99.7%]	91.7% (22/24)	[75.1 %; 98.2%]	93.1% (67/72)	[88.2 %; 96.0%]
Positive Sample	**DNA Input**	**PPA**		**PPA**		**PPA**		**PPA**	
		% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90% CI
LP	High	100% (6/6)	[78.51 %; 100%]	100% (6/6)	[78.51 %; 100%]	100% (6/6)	[78.51 %; 100%]	100% (18/18)	[91.64 %; 100%]
	Low	100% (6/6)	[78.51 %; 100%]	100% (6/6)	[78.51 %; 100%]	100% (6/6)	[78.51 %; 100%]	100% (18/18)	[91.64 %; 100%]
HP	High	100% (6/6)	[78.51 %; 100%]	100% (6/6)	[78.51 %; 100%]	100% (6/6)	[78.51 %; 100%]	100% (18/18)	[91.64 %; 100%]
	Low	100% (6/6)	[78.51 %; 100%]	100% (6/6)	[78.51 %; 100%]	100% (6/6)	[78.51 %; 100%]	100% (18/18)	[91.64 %; 100%]
All Positives		100% (24/24)	[86.4 %; 100%]	100% (24/24)	[86.4 %; 100%]	100% (24/24)	[86.4 %; 100%]	100% (72/72)	[97.8 %; 100%]
All Samples		**Agreement**		**Agreement**		**Agreement**		**Agreement**	
		% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90% CI
Overall		95.8 % (46/48)	[90.4 %; 98.3%]	97.9% (47/48)	[93.3 %; 99.4%]	95.8% (46/48)	[90.4 %; 98.3%]	96.5% (139/144)	[94.0 %; 98.0%]

Table 8.1.5 Interlaboratory Reproducibility per Sample Presented as Overall and by Day

	Bladder EpiCheck Result				
	Timepoint (Day)			**Overall**	
	1	**2**	**3**		

Negative Sample	DNA Input	NPA		NPA		NPA		NPA		Invalid Rate	
		% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90 % CI
LN	High	100 % (6/6)	[78.51% ; 100 %]	100 % (6/6)	[78.51%; 100%]	100% (6/6)	[78.51%; 100%]	100% (18/18)	[91.64% ; 100%]	0	--
	Low	100 % (6/6)	[78.51% ; 100 %]	100 % (6/6)	[78.51%; 100%]	100% (6/6)	[78.51%; 100%]	100% (18/18)	[91.64% ; 100%]		
HN	High	100 % (6/6)	[78.51% ; 100 %]	66.7 % (4/6)	[40.94%; 85.23 %]	100% (6/6)	[78.51%; 100%]	88.9% (16/18)	[75.99% ; 95.29%]	5.3% (2/38)	[2.2 %; 12.0 %]
	Low	100 % (4/4)	[70.89% ; 100 %]	75% (6/8)	[52.37%; 89.11 %]	83.3% (5/6)	[57.47%; 94.87 %]	83.3% (15/18)	[69.42% ; 91.68%]		
All Negatives		100 % (22/22)	[85.3%; 100 %]	84.6 % (22/26)	[67.6 %; 94.1 %]	95.8% (23/24)	[80.4 %; 99.7 %]	93.1% (67/72)	[88.2%; 96.0%]	2.7% (2/74)	[1.1 %; 6.3 %]
Positive Sample	DNA Input	PPA		PPA		PPA		PPA		Invalid Rate	
		% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90 % CI
LP	High	100 % (6/6)	[78.51% ; 100 %]	100 % (6/6)	[78.51%; 100%]	100% (6/6)	[78.51%; 100%]	100% (18/18)	[91.64% ; 100%]	0	--
	Low	100 % (6/6)	[78.51% ; 100 %]	100 % (6/6)	[78.51%; 100%]	100% (6/6)	[78.51%; 100%]	100% (18/18)	[91.64% ; 100%]		
HP	High	100 % (6/6)	[78.51% ; 100 %]	100 % (6/6)	[78.51%; 100%]	100% (6/6)	[78.51%; 100%]	100% (18/18)	[91.64% ; 100%]	2.7% (1/37)	[0.8 %; 8.6 %]
	Low	100 % (6/6)	[78.51% ; 100 %]	100 % (5/5)	[75.27%; 100%]	100% (7/7)	[81.00%; 100%]	100% (18/18)	[91.64% ; 100%]		
All Positives		100 % (24/24)	[86.4%; 100 %]	100 % (23/23)	[85.9 %; 100%]	100% (25/25)	[86.9 %; 100%]	100% (72/72)	[97.8%; 100%]	1.4% (1/73)	[0.4 %; 4.5 %]
All Samples		Agreement		Agreement		Agreement		Agreement		Invalid Rate	
		% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90% CI	% (n/N)	90 % CI
Overall		100 %	[96.6%;	91.8 %	[85.4 %;	98.0% (48/49)	[93.4 %;	96.5% (139/144)	[94.0%; 98.0%]	2.0% (3/14	[1.0 %;

	(46/46)	100 %]	(45/49)	95.6 %]		99.4 %]			7)	4.1 %]

Operator-to-Operator/Day-to-Day

An operator-to-operator and day-to-day repeatability and reproducibility study was performed to assess the within-run, between-run, and between-operator variation of Bladder EpiCheck when performed by different operators over 5 non-consecutive days. Healthy donor urine pools were spiked with bladder cancer-derived cell line material at different input levels to formulate 4 test samples spanning the measurable range of EpiScore values. Overall performance characteristics (agreement, PPA, and NPA) are summarized in Tables 8.1.6, 8.1.7, and 8.1.8 below. There were no invalid results and 1 control (plate) failure out of 21 plates (1/21 = 4.8% [95% CI 1.1%; 18.8%].

Table 8.1.6 Within Site Precision per Sample Presented as Overall and by Day (5 Days)

Sample Category	**Bladder EpiCheck Result**											
	Timepoint (Day)										**Overall**	
	1		**2**		**3**		**4**		**5**			
Negative Sample	**NPA**		**NPA**		**NPA**		**NPA**		**NPA**		**NPA**	
	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95% CI
LN	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (50/50)	[94.9%; 100%]
HN	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	90 % (9/10)	[59.5%; 99.3 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	98% (49/50)	[91.5%; 99.6%]
All Negatives	100 % (20/20)	[83.9%; 100 %]	100 % (20/20)	[83.9%; 100 %]	95 % (19/20)	[76.4%; 99.1 %]	100 % (20/20)	[83.9%; 100 %]	100 % (20/20)	[83.9%; 100 %]	99% (99/100)	[94.6%; 99.8%]
Positive Sample	**PPA**		**PPA**		**PPA**		**PPA**		**PPA**		**PPA**	
	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95% CI
LP	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	90 % (9/10)	[59.5%; 99.3 %]	98% (49/50)	[91.5%; 99.6%]
HP	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (50/50)	[94.9%; 100%]
All Positives	100 % (20/	[83.9%; 100	100 % (20/	[83.9%; 100	100 % (20/	[83.9%; 100	100 % (20/	[83.9%; 100	95 % (19/	[76.4%; 99.1	99% (99/100)	[94.6%; 99.8%]

	20)	%]	20)	%]	20)	%]	20)	%]	20)	%]		
All Samples	**Agreement**		**Agreement**		**Agreement**		**Agreement**		**Agreement**		**Agreement**	
	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95% CI
Overall	100 % (40/40)	[91.2%; 100 %]	100 % (40/40)	[91.2%; 100 %]	97.5% (39/40)	[87.1%; 99.6 %]	100 % (40/40)	[91.2%; 100 %]	97.5% (39/40)	[87.1%; 99.6 %]	99% (198/200)	[96.4%; 99.7%]

Table 8.1.7 Within Site Precision per Sample Presented as – Overall and by Operator 1 per Day (5 Days)

Sample Category	**Bladder EpiCheck Result**											
	Operator 1										**Overall**	
	Timepoint 1		**Timepoint 2**		**Timepoint 3**		**Timepoint 4**		**Timepoint 5**			
Negative Sample	**NPA**		**NPA**		**NPA**		**NPA**		**NPA**		**NPA**	
	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95% CI
LN	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100% (25/25)	[86.7%; 100%]
HN	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100% (25/25)	[86.7%; 100%]
All Negatives	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100% (50/50)	[94.9%; 100%]
Positive Sample	**PPA**		**PPA**		**PPA**		**PPA**		**PPA**		**PPA**	
	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95% CI
LP	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	80 % (4/5)	[37.6%; 95.4 %]	96% (24/25)	[80.5%; 99.7%]
HP	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (25/25)	[86.7%; 100%]
All Positives	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	90 % (9/10)	[59.5%; 99.3 %]	98% (49/50)	[91.5%; 99.6%]

All Samples	Agreement		Agreement		Agreement		Agreement		Agreement		Agreement	
	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95% CI
Overall	100 % (20/20)	[83.9%; 100 %]	100 % (20/20)	[83.9%; 100 %]	100 % (20/20)	[83.9%; 100 %]	100 % (20/20)	[83.9%; 100 %]	95 % (19/20)	[76.4%; 99.1 %]	99% (99/100)	[94.6%; 99.8%]

Table 8.1.8 Within Site Precision per Sample Presented as – Overall and by Operator 2 per Day (5 Days)

Sample Category	Bladder EpiCheck Result											
	Operator 2										Overall	
	Timepoint 1		Timepoint 2		Timepoint 3		Timepoint 4		Timepoint 5			
Negative Sample	NPA		NPA		NPA		NPA		NPA		NPA	
	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95% CI
LN	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (25/25)	[86.7%; 100%]
HN	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	80 % (4/5)	[37.6%; 95.4 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	96% (24/25)	[80.5%; 99.7%]
All Negatives	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	90 % (9/10)	[59.5%; 99.3 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	98% (49/50)	[91.5%; 99.6%]
Positive Sample	PPA		PPA		PPA		PPA		PPA		PPA	
	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95 % CI	% (n/N)	95% CI
LP	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (25/25)	[86.7%; 100%]
HP	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (5/5)	[56.6%; 100 %]	100 % (25/25)	[86.7%; 100%]
All Positives	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (10/10)	[78.7%; 100 %]	100 % (50/50)	[94.9%; 100%]
All Samples	Agreement		Agreement		Agreement		Agreement		Agreement		Agreement	
	%	95	%	95	%	95	%	95	%	95	%	95% CI

	(n/N)	% CI	(n/N)	% CI	(n/N)	% CI	(n/N)	% CI	(n/N)	% CI	(n/N)	
Overall	100 % (20/20)	[83.9%; 100 %]	100 % (20/20)	[83.9%; 100 %]	95 % (19/20)	[76.4%; 99.1 %]	100 % (20/20)	[83.9%; 100 %]	100 % (20/20)	[83.9%; 100 %]	99% (99/100)	[94.6%; 99.8%]

Lot-to-Lot/Instrument-to-Instrument

A lot-to-lot and instrument-to-instrument repeatability and reproducibility study was performed to assess the variability of Bladder EpiCheck when performed using 3 unique kit lots and 3 unique PCR instruments. Healthy donor urine pools were spiked with bladder cancer-derived cell line material at different DNA concentration levels to create 4 test samples spanning the range of EpiScore values. A minimum of 70 replicates were tested for each sample type. Overall agreement is summarized in Table 8.1.9 below.

Table 8.1.9 Within Site Precision – Overall and by Lot, Instrument, and EpiScore Value

Condition	% (n/N)	Wilson Score 95% CI
Overall	100.0% (294/294)	99.09%; 100.0%
Lot		
1	100.0% (175/175)	98.48%; 100.0%
2	100.0% (59/59)	95.62%; 100.0%
3	100.0% (60/60)	95.69%; 100.0%
Instrument		
1	100.0% (214/214)	98.75%; 100.0%
2	100.0% (40/40)	93.66%; 100.0%
3	100.0% (40/40)	93.66%; 100.0%
EpiScore Value		
Low Negative	100.0% (74/74)	96.47%; 100.0%
High Negative	100.0% (75/75)	96.52%; 100.0%
Low Positive	100.0% (70/70)	96.28%; 100.0%
High Positive	100.0% (75/75)	96.52%; 100.0%

8.2 Analytical Sensitivity

Limit of Blank (LoB)

A Limit of Blank study was conducted to determine the residual levels of non-specific background signal in the absence of template DNA. Sixty (60) replicates of non-template control (without DNA) were tested across 2 reagent lots – a total of 120 test results. Mean Cq results were 44.75 and 44.86 for Lots 1 and 2, respectively. Using a non-parametric approach, the resulting LoB was determined to be a Cq value equal to 45.

Functional Limit of Detection (fLoD)

The functional Limit of Detection (fLoD) refers to the lowest total DNA that is

required for the assay and was determined by testing DNA extracted from positive clinical specimens mixed with DNA extracted from pooled healthy individuals (negative clinical specimens) to create a clinical specimen with an EpiScore near the clinical cutoff (Low Positive). This sample was tested across a range of 5 concentrations (two-fold dilutions) around and below the assay cut-off (1.2 ng/well, 0.65 ng/well, 0.30 ng/well, 0.15 ng/well, and 0.075 ng/well). A blank sample (0 ng/well) was also tested. Each concentration was tested in replicates of 30 using one lot of reagents and one instrument – a total of 330 test measurements. The fLoD was determined from a PROBIT regression model as the concentration at which, with 95% probability and 90% CI, the measurement result yielded a "positive" classification. Hit Rate and PROBIT results are summarized in Tables 8.2.1 and 8.2.2, respectively. Using this approach, the estimated fLoD was determined to be 0.186 ng/well (2.23 ng/sample).

Table 8.2.1 Hit Rate

DNA Conc (ng/PCR well)	**Detection** (n/N)	**Hit Rate** %	**90% Confidence Interval**	
			Lower	**Upper**
0.84	30/30	100	94.8%	100%
0.42	30/30	100	94.8%	100%
0.27	30/30	100	94.8%	100%
0.15	26/30	86.7	76.8%	92.7%
0.075	17/30	56.7	45.0%	67.6%

Table 8.2.2 Probability Estimated from the PROBIT Model

Probability	**Sample Type**	**Concentration** (ng/well)	**90% Confidence Intervals**	
			Lower	**Upper**
0.95	Clinical	0.186	0.88	0.98
	Contrived	0.202	0.89	0.98
0.75	Clinical	0.104	0.65	0.83
	Contrived	0.081	0.63	0.85
0.5	Clinical	0.070	0.35	0.65
	Contrived	0.043	0.28	0.72
0.25	Clinical	0.047	0.10	0.47
	Contrived	0.023	0.06	0.59

Tumor Limit of Detection (tLoD)

The Tumor Limit of Detection (tLoD) refers to the minimum amount of tumor DNA in the lowest total volume of DNA that can be detected and was determined by testing DNA extracted from bladder cancer positive patient specimens and mixing with DNA extracted from healthy urine donor samples in a ratio gradient of 7 positive DNA spike-in levels (30%, 20%, 15%, 12%, 8%, and 4%). These samples were tested using 2 reagent lots in 30 replicates total. Samples with 100%, 50% and 0% positive DNA spike-ins were also tested, but only in 10 replicates total. The total DNA concentration was kept constant at 1-2x fLoD. The tLoD was calculated from a PROBIT regression model as the concentration at which with 95% probability the measurement result yields a "positive" classification. Hit rate and PROBIT estimation results are summarized in Tables

8.2.3 and 8.2.4, respectively. Based on this model, the tLoD was determined to be 7.5%, which equates to a minimum amount of tumor DNA in a clinical sample of ~0.17 ng.

Table 8.2.3 Hit Rate

Tumor DNA Fraction (%)	Hit Rate (n/N)	Hit Rate (%)	90% Confidence Interval	
			Lower	Upper
100	10/10	100	85.9%	100%
50	10/10	100	85.9%	100%
30	30/30	100	94.8%	100%
20	30/30	100	94.8%	100%
15	30/30	100	94.8%	100%
12	30/30	100	94.8%	100%
8	29/30	96.7	89.5%	99.0%
4	11/30	36.7	26.4%	48.4%
0	0/10	0	0%	14.1%

Table 8.2.4 Probability Estimated from the PROBIT Model

Probability	Sample Type	Tumor DNA Fraction (%)	90% Confidence Limits	
			Lower	Upper
0.95	Clinical	7.5	85.0%	99.0%
	Contrived	12.7	89.0%	98.0%

Methylation Limit of Detection (mLoD)

The Methylation Limit of Detection (mLoD) refers to the minimal detectable level of methylation across the assay's target loci and was determined using a mixture of methylated and unmethylated artificial plasma DNA to create a gradient of different levels of methylation. This study took place across 2 phases, performed using contrived DNA samples.

In Study 1 (Phase I), a preliminary mLoD was established using a single PCR mix (Mix A, which consisted of biomarkers BE-1 and BE-2), as it represented the lower end of the sensitivity range of the assay. A gradient of 10 admixtures (10%, 5%, 2.5%, 1%, 0.5%, 0.25%, 0.1%, 0.05%, 0.025%, and 0.01%), along with a 0% methylation sample, were tested using one reagent lot and between 16 and 60 replicates, depending on the methylation level – a total of 378 total measurements. Undigested 100% methylated UC was also run as a positive control. Hit rate and PROBIT results for both biomarkers are summarized in Tables 8.2.5 and 8.2.6, respectively. Based on interpolation from the PROBIT estimation, the higher of the two mLoD values was used as the preliminary mLoD: 0.348%.

In Study 2 (Phase II), the preliminary mLoD value determined in Phase I was confirmed for the other 13 biomarkers in the assay using a subset of the admixture gradients used in Phase I (3 methylation levels around the preliminary LoD: 0.05%, 0.1%, and 0.25%) and tested using one reagent lot and 60 replicates per methylation level. Undigested 100% methylated UC was also run as a positive

control. Hit rate results for the other 13 biomarkers are summarized in Table 8.2.7 below. Based on the results, biomarkers BE-4 through BE-15 had hit rates of 100.0% at values below the preliminary mLoD. The one exception, BE-3, underwent a follow-up root analysis, and preparation of mix B (containing biomarkers BE-3 and BE-4) was repeated across the entire range of tested ratios from Phase II. Results from the repeat preparation showed acceptable hit rates below the preliminary mLoD.

Table 8.2.5 Hit Rate with Upper One-Sided 95% Confidence Interval – per Marker and Methylation % (Phase I)

Marker	Methylation (%)	Hit Rate % (n/N)	Upper One-Sided 95% CL
BE-1	0.01	10.00% (3/30)	25.62%
	0.025	10.00% (3/30)	25.62%
	0.05	6.67% (4/60)	15.93%
	0.1	40.00% (24/60)	52.63%
	0.25	65.00% (39/60)	75.83%
	0.5	100.0% (30/30)	100.0%
	1	100.0% (30/30)	100.0%
	2.5	100.0% (16/16)	100.0%
	5	100.0% (16/16)	100.0%
	10	100.0% (16/16)	100.0%
BE-2	0.01	10.00% (3/30)	25.62%
	0.025	76.67% (23/30)	88.21%
	0.05	78.33% (47/60)	86.88%
	0.1	98.33% (59/60)	99.71%
	0.25	98.33% (59/60)	99.71%
	0.5	100.0% (30/30)	100.0%
	1	100.0% (30/30)	100.0%
	2.5	100.0% (16/16)	100.0%
	5	100.0% (16/16)	100.0%
	10	100.0% (16/16)	100.0%

Table 8.2.6 Probability Estimated from PROBIT Model

Marker	Probability	Methylation %	95% Fiducial Limits
BE-1	0.90	0.34872	0.30102; 0.42287
BE-2	0.90	0.06681	0.05337; 0.09068

Table 8.2.7 Hit Rate with Upper One-Sided 95% Confidence Limit – Per Marker and per Methylation % (Phase II)

Marker	Methylation %	Hit Rate % (n/N)	Upper One-Sided 95% CL
BE-3	0.05	39.17% (47/120)	46.66%
	0.1	39.17% (47/120)	46.66%
	0.25	56.90% (66/116)	64.22%
BE-4	0.05	53.33% (32/60)	63.55%
	0.1	78.33% (47/60)	85.76%

	0.25	98.21% (55/56)	99.60%
BE-5	0.05	35.00% (21/60)	45.58%
	0.1	80.00% (48/60)	87.11%
	0.25	96.43% (54/56)	98.81%
BE-6	0.05	47.50% (57/120)	54.97%
	0.1	75.83% (91/120)	81.65%
	0.25	92.24% (107/116)	95.43%
BE-7	0.05	50.00% (30/60)	60.39%
	0.1	70.00% (42/60)	78.69%
	0.25	98.21% (55/56)	99.60%
BE-8	0.05	46.67% (28/60)	57.17%
	0.1	70.00% (42/60)	78.69%
	0.25	94.44% (51/54)	97.76%
BE-9	0.05	10.00% (6/60)	18.19%
	0.1	36.67% (22/60)	47.27%
	0.25	91.07% (51/56)	95.59%
BE-10	0.05	26.67% (16/60)	36.91%
	0.1	41.67% (25/60)	52.27%
	0.25	87.50% (49/56)	93.08%
BE-11	0.05	71.67% (41/60)	80.14%
	0.1	91.67% (55/60)	95.86%
	0.25	100.0% (55/55)	100.0%
BE-12	0.05	48.33% (29/60)	58.79%
	0.1	88.33% (53/60)	93.55%
	0.25	100.0% (56/56)	100.0%
BE-13	0.05	65.00% (39/60)	74.28%
	0.1	93.33% (56/60)	96.97%
	0.25	100.0% (56/56)	100.0%
BE-14	0.05	50.85% (30/59)	61.28%
	0.1	73.33% (44/60)	81.57%
	0.25	100.0% (56/56)	100.0%
BE-15	0.05	48.28% (28/58)	58.90%
	0.1	81.67% (49/60)	88.45%
	0.25	96.43% (54/56)	98.81%

8.3 Analytical Specificity

Digestion Restriction Efficiency

Digestion restriction efficiency was established by evaluating the digestion of all 15 Bladder EpiCheck markers using 100% unmethylated synthetic (plasmid) DNA tested across a series of dilution levels for a total of 30 replicates based on the relationship of the ΔCq between the non-digestible IR marker (100% copies in the reaction) and digested targets (BE markers 1-15). Digestion efficiency for all 15 markers was shown to exceed 99.9%, with an average efficiency of 99.997%.

An additional study was performed to assess the impact of hemi-methylated

molecules have on the enzymatic digestion step, as well as the contamination risk, in the Bladder EpiCheck assay protocol performed using synthetic DNA molecules of a representative locus (BE-14) containing all different permutations of the targeted cut site. Results showed that hemi-methylated DNA does represent a digestion efficiency profile similar to unmethylated DNA; however, at best, this reflects a very small sub-population of target molecules, so the effect should be negligible.

Digestion Restriction Efficiency – Exclusivity
Digestion restriction efficiency (Exclusivity) was established by evaluating the digestion of nonmethylated markers to avoid false positive results using a commercially available hypo-methylated human genomic DNA sample (<5% average global methylation), added to the digestion reaction at an extreme excess (2.5ug/reaction), and tested in 10 replicates (with 6 replicates of a control reaction with no digestion enzyme to reflect the full amplification between test and control markers). The digestion efficiency was greater than 99% for 9 of the 15 biomarkers, ensuring that no non-specific signal is generated in the qPCR step.

A supplementary meta-analysis was conducted to demonstrate that each of the 15 Bladder EpiCheck markers can be digested at or above the 99.9% level. Raw data from 2,300 clinical specimens from US and European cohorts (including a wide range of methylation levels at all 15 loci) was analyzed, and max digestion was determined for each biomarker. The Max digestion percentage was >99.99% for all 15 biomarkers, thus further ensuring that no non-specific signal is generated in the qPCR step.

Primer Probe Specificity
In-Silico PCR analysis for each locus (Informative markers BE-1 to BE-15 and the Internal Reference (IR)) obtained a single hit using the forward and reverse primer sequences. Additionally, confirmatory PCR was performed using each primer pair, followed by gel electrophoresis. This showed only one product amplified per primer pair, this confirming the in-silico analysis.

8.4 Robustness (Guardbanding)

Robustness of the Bladder EpiCheck was evaluated using 4 contrived specimens (T24 cell line DNA spiked into DNA extracted from healthy volunteer urine pools) representing the full range of EpiScore values (LN, HN, LP, and HP). Each sample was run in quintuplicates across a series of 13 conditions with variations to PCR denaturation and annealing temperatures, digestion temperature, and/or digestion time. Overall agreement was shown to be 98.5% [96.77%; 99.31%].

A supplementary study was conducted to further demonstrate the robustness of the Bladder EpiCheck assay using 4 clinical specimens (DNA extracted from clinical specimens adjusted using DNA extracted from healthy urine donor pools) that represent the full range of EpiScore values (LN, HN, LP, and HP). Each sample was tested in quintuplicates across 5 different assay conditions

(including the control condition). Overall agreement was shown to be 99.3% [96.9%; 99.8%], thus confirming the robustness of the assay.

8.5 Interference

Performance of Bladder EpiCheck was evaluated with 27 potentially interfering substances (including common urine constituents, microbial contaminants, therapeutic agents, and laboratory preservatives), which were tested across 3 sample pools (2 negative pools from healthy individuals, and one positive pool of healthy urine spiked with bladder cancer cell-line material) at 2 concentrations, with each sample run in 3 replicates. Preliminary results suggested that uric acid and ampicillin were both potential interferents, so a dose response analysis was conducted, which showed that interference either did not repeat itself or was present at elevated levels that are not clinically meaningful. Therefore, no evidence of interference caused by the substances tested at clinically relevant physiological ranges exists.

8.6 Real-Time and In-Use Stability

In-use and real-time stability testing of 3 lots of the Bladder EpiCheck kit stored at the recommended storage condition of -20°C/2-8°C/25°C (depending on the reagents storage specifications) was conducted at six separate time points. Each lot was tested using 4 contrived urine pools representing the full range of EpiScore values (LN, HN, LP, and HP) tested in 7 replicates at each timepoint. For in-use stability assessment, during each time point, all kits from the same lot (n=3) underwent the same freeze/thaw and handling procedures. For real-time stability assessment, at each time point, “fresh” (unused) Extraction and test kits were used. Each “run” included the Undigested (UC) and Non-Template (NTC) Controls. For T=0, a total of 84 samples were tested (4 sample pools tested in each of 3 kit lots in replicates of 7. For T=1 through T=5, a total of 168 samples were tested (4 samples pools tested in each of 3 kit lots in replicates of 7 spanning both studies). Results showed that there was no significant change in performance when testing kits and controls up to 486 days.

8.7 Freeze-Thaw Stability

Freeze/Thaw stability testing of three lots of the Bladder EpiCheck Test kit stored at the recommended storage condition of -20°C was conducted at three separate test points (T = 1, 8 and 12 cycles). Each lot was tested using 4 contrived urine pools (T24 cell line DNA spiked into DNA extracted from healthy volunteer urine pools) representing the full range of EpiScore values (LN, HN, LP, and HP) with 5 replicates per sample. Results showed no significant performance changes and low variability in EpiScore value between the 3 timepoints. The stability claim of 10 cycles was then confirmed in a supplementary study using 4 clinical samples representing the full range of EpiScore values (LN, HN, LP, and HP) with 5 replicates per sample over 2 timepoints (1 cycle and 12 cycles).

8.8 Shipping Stability

12 Extraction Kit boxes and 12 Test kit boxes will be used to evaluate the packing

tolerance due to transportation (sudden changes in temperature for 24 hours, vibration, pressure, and drop tolerance). From the 12 boxes, two boxes will be used to evaluate the functionality of the kits (i.e., extracting DNA and prepare the DNA for the PCR analysis) and to test the performance of the kit (quantitative and qualitative results). In addition, a single control box (i.e., test kit that won't go through any transportation simulation) will be used as control. The performance of the kit will be tested on urine samples collected from 4 healthy and 4 Bladder Cancer (n=8) subjects. Each sample will be tested in five replicates by the kits underwent the transportation analysis (n=40) and in duplicate by the control kit (n=16). Overall, Positive, and Negative agreement were 100%.

8.9 Sample Stability

30 clinical specimens, collected from 15 bladder cancer patients and 15 normal donors across the full range of EpiScore values, were divided into 3 parts and used to evaluate the stability of fresh urine, urine pellet, and extracted DNA. Each sample was tested immediately and after storage. Each clinical sample was divided into different aliquots and tested at 3 timepoints depending on the specimen type (Fresh urine: 0, 5, and 6 days; Pelleted urine: 0, 14, and 21 days; DNA: 0, 30, and 60 days) after storage at the recommended conditions. Agreement results are shown in Tables 8.9.1, 8.9.2, and 8.9.3 for urine, pelleted urine, and DNA, respectively. Results confirm a stability claim of 5 days for fresh urine at 2-8ºC, urine pellets for 19 days at -20ºC, and DNA for 30 days at -20ºC.

Table 8.9.1 Bladder EpiCheck Results, Overall and by Timepoint – Fresh Urine

Timepoint	EpiCheck Result	
	% (n/N)	Wilson Score 95% CI
Overall	99.01% (100/101)	95.68%; 99.78%
0 Days (T_0)	100% (34/34)	92.63%; 100%
5 Days (T_1)	100% (33/33)	92.42%; 100%
6 Days (T_2)	97.06% (33/34)	87.84%; 99.34%

Table 8.9.2 Bladder EpiCheck Results, Overall and by Timepoint – Urine Pellet

Timepoint	EpiCheck Result	
	% (n/N)	Wilson Score 95% CI
Overall	100.0% (90/90)	97.08%; 100.0%
0 Days (T_0)	100.0% (30/30)	91.73%; 100.0%
14 Days (T_1)	100.0% (30/30)	91.73%; 100.0%
21 Days (T_2)	100.0% (30/30)	91.73%; 100.0%

Table 8.9.3 Bladder EpiCheck Results, Overall and by Timepoint - Extracted DNA

Timepoint	EpiCheck Result	
	% (n/N)	Wilson Score 95% CI
Overall	98.25% (112/114)	94.84%; 99.42%
0 Days (T_0)	100.0% (38/38)	93.35%; 100.0%
30 Days (T_1)	100.0% (38/38)	93.35%; 100.0%

60 Days (T_2)	94.87% (36/38)	85.28%; 98.24%

8.10 DNA Extraction Efficiency

Forty-seven clinical urine specimens, collected from bladder cancer patients and healthy donors, were used for this study. Each specimen was pelleted, split into 10mL urine equivalents, and DNA was extracted from each split specimen using 4 Bladder EpiCheck Extraction lots. Performance of the Bladder EpiCheck kits was evaluated by invalid rate and Bladder EpiCheck test performance and tested in the Bladder EpiCheck test using one ABI 7500 Fast Dx Real-Time PCR system and analyzed using Bladder EpiCheck software (Ver. 1.9.19). A total of 1 sample failed after extraction, leading to an overall sample failure rate of 2.1%. Study results showed an overall, positive, and negative agreement of 100%, justifying that the Bladder EpiCheck DNA Extraction Kit met the extraction capabilities for use with the Bladder EpiCheck test.

9. Performance Testing (Clinical)

9.1 Clinical Cutoff (Training and Feasibility Data)

The assay cut-off and proprietary EpiScore algorithm for the Bladder EpiCheck software were validated on a set of urine samples collected from control patients with a history of bladder cancer and bladder cancer positive patients confirmed by cystoscopy and pathology. The total sample size for the software algorithm development included 178 samples which were collected in two separate sets. The first set of samples served for the cut-off definition. The set included 109 samples, 40 of which were collected from control patients with a history of bladder cancer, and 69 collected from Urothelial Cell Carcinoma (UCC) positive patients confirmed by pathology. The second samples set served for the validation of the cut-off. The set included 67 samples, of which 51 were collected from control patients with a history of bladder cancer, and 16 collected from UCC positive patients confirmed by pathology. The derived Bladder EpiCheck algorithm sensitivity and specificity compared to pathology outcome demonstrated a sensitivity of 94% for UCC recurrence and specificity of 84%.

9.2 Method Comparison

A multi-center, prospective, IRB-approved longitudinal study was conducted at 11 academic and urology specialty medical centers in the U.S. and Canada to establish the performance of Bladder EpiCheck as compared to the predicate device (UroVysion Bladder Cancer Recurrence Kit). Performance of the Bladder EpiCheck kit was also assessed to a gold standard (GS) using both clinical cytology and combined cystoscopy/pathology data.

A total of 674 subject were enrolled in the study. Eligible subjects were any male or female subject aged 22 and above, diagnosed with incident or recurrent UCC and undergoing surveillance for bladder cancer recurrence, who had urothelial cell carcinoma tumor resected within 12 months from baseline visit and had a plan for cystoscopy surveillance. The study included up to 3 visits: baseline and

up to 2 additional surveillance visits, where voided urine specimens were collected. In total, voided urine specimens were collected from 583 subjects. For subjects who experienced a recurrence (as determined by positive cytology /pathology), the first positive visit was used (i.e., the visit at which the diagnosis of recurrence was established). For the non-recurring subjects, the last negative visit was used for subjects with multiple surveillance visits.

DNA extracted from urine specimens was tested using the Bladder EpiCheck test and compared to the previously discussed gold standard (GS). Classification by the GS was as follows: a subject was considered "positive" if the interpretation for either cytology or the combined cystoscopy/pathology results were positive, and a subject was considered "negative" if both cytology and the combined cystoscopy/pathology results were negative. In total, valid Bladder EpiCheck and GS results were obtained from 449 subjects. The Bladder EpiCheck test performed with an accuracy of 78.8% ([74.8%; 82.4%]), a sensitivity of 66.7% ([58.4%; 74.0%]), and a specificity of 84.2% ([79.8%; 87.9%]). Further details of the performance characteristics of the Bladder EpiCheck test compared to the established GS is found in Table 9.2.1. Additionally, performance of the Bladder EpiCheck test was evaluated in several subgroup analyses, including cancer grade (High, Low, No grade) and stage (CIS, T1, T2, Ta). Performance results from these analyses were comparable to the overall performance characteristics of the Bladder EpiCheck test.

Table 9.2.1 Bladder EpiCheck Performance vs. Gold Standard

	Gold Standard Result		
	Negative	**Positive**	**Total GS**
	N	**N**	**N**
EpiCheck Result			
Negative	262	46	308
Positive	49	92	141
Total EpiCheck	311	138	449
Accuracy	78.8% [74.8%; 82.4%] (354/449)		
Sensitivity	66.7% [58.4%; 74.0%] (92/138)		
Specificity	84.2% [79.8%; 87.9%] (262/311)		
PPV	65.3% [57.1%; 72.6%] (92/141)		
NPV	85.1% [80.7%; 88.6%] (262/308)		

Performance of the Bladder EpiCheck test was also compared to the predicate device with respect to the GS sample definition. In total, valid Bladder EpiCheck, UroVysion, and GS results were obtained from 352 samples. Based on the results (Table 9.2.2), the Bladder EpiCheck was slightly more sensitive (+4.82%) and slightly less specific (-2.97%) than the predicate device, thus showing that the Bladder EpiCheck test was non-inferior to the predicate. An additional analysis was performed by cancer grade, which showed better sensitivity (+5.00%) and similar specificity (0.00%) between the two assays.

Table 9.2.2. Bladder EpiCheck and UroVysion vs. Gold Standard – Matched Cases

Gold Standard Result	Bladder EpiCheck Result				
	Negative		Positive		Total
	UroVysion Result		UroVysion Result		
	Negative	Positive	Negative	Positive	N
Negative	195	18	26	30	269
Positive	21	8	12	42	83
Total	216	26	38	72	352

Metric		% (n/N)	95% CI
Sensitivity	**EpiCheck**	65.1% (54/83)	54.8%; 75.3%
	Urovysion	60.2% (50/83)	49.7%; 70.8
	Difference	4.82%	-5.7%; 15.3%
Specificity	**EpiCheck**	79.2% (213/269)	74.3%; 84.0%
	Urovysion	82.2% (221/269)	77.6%; 86.7%
	Difference	-2.97%	-7.8%; 1.9%

9.3 Specificity in Urology Patients without Bladder Cancer

A multi-center, prospective study was conducted to establish the specificity of Bladder EpiCheck test in urology patients without prior history or clinical evidence of bladder cancer. The study population consisted of 147 subjects, aged 22 and above, visiting the urology clinic for any non-bladder cancer disorders. Subjects were excluded from the study if they had known current or prior diagnosis of both invasive and non-muscle invasive bladder cancer. The specificity of Bladder EpiCheck in this study population was 98.0%. Covariates analysis by sex, age group, ethnicity, race, smoking status, reason for visit at clinic, non-cancer genitourinary disease type, presence of hematuria, BPH, UTI, and STD were performed. No statistical significance was found, indicating that there was no impact of any of the tested covariates on the applicability of the primary analysis results on the population tested.

9.4 Clinical Specificity – Cross Reactivity with Other Cancers

The effect of potential cross-reactivity was assessed using banked remnant de-identified urine samples collected from patients diagnosed with different types of cancers, specifically cancers that are more likely to shed cancer cells into the bladder such as renal cell carcinoma (RCC) and prostate cancer as well as other solid and hematology cancer. 147 urine samples were assessed. The most frequent cancer category was genitourinary, with 54 subjects (37%) including prostate and renal cell carcinoma, followed by gastrointestinal with 26 subjects (18%); 15 subjects (10%) had gynecological cancers and 10 subjects had hematological cancers (7%). 42 subjects (29%) had other solid cancer types. The study population had a mean age of 65 years old (ranging from 27 to 89) with a similar rate of sexes. Nearly all subjects were non-smokers or former smokers, with 12% current smokers. In this study, the specificity was found to be 95% (140/147: Prostate (3), RCC (2), Uterine (1), pancreas (1)) (95% confidence interval of 90.4%-98.1%), confirming the high specificity of Bladder EpiCheck in patients

with cancer other than bladder cancer.

10. Conclusion

The analytical and clinical performance supports that the Nucleix EpiCheck Test is substantially equivalent with the predicate device.