AssureMDx™
Patient Result Report

Vesica Health®
Noninvasive. Early. Detection.

Patient Name:	Specimen ID#:	Physician:
Date of Birth:	Collection Date:	Client Name:
Sex:	Received Date:	Client Address:
Sample Type:	Report Date:	
MRN/Patient#:	Vesica Accession#:	

Patient Test Result: Positive

A positive AssureMDx test result is associated with a significantly increased risk of urothelial carcinoma. In clinical validation, the post-test cancer probability ranged from 31% to 42%, corresponding to a two- to eight-fold increase in risk compared to baseline, depending on hematuria type.[1-2]

Clinical Interpretation

AssureMDx evaluates six validated molecular biomarkers to identify DNA alterations associated with urothelial carcinoma. The assay integrates these biomarker results with hematuria type to generate a patient-specific molecular risk profile for bladder and upper tract urothelial carcinoma. Performance has been validated in prospective multi-center cohorts demonstrating high sensitivity and negative predictive value for urothelial carcinoma detection.[1–4]

A ***positive test result*** indicates the presence of one or more molecular alterations associated with tumor development or progression and should be interpreted as an increased likelihood of urothelial carcinoma. A negative test result indicates all biomarkers are within normal parameters and supports a very low likelihood of malignancy. These findings are intended to assist clinicians in determining the appropriate timing and need for urologic evaluation based on each patient's presentation and risk factors and may warrant expedited urologic evaluation, particularly in patients who might otherwise be managed conservatively.

Biomarker Status:

Biomarker	Category	Functional Role	Clinical Relevance	Result
TWIST1 [5-7]	DNA methylation	EMT / invasion regulator	**When positive, indicates aggressive tumor biology with promoter hypermethylation significantly associated with progression, stage, grade, and recurrence potential.**	Positive
OTX1 [8-10]	DNA methylation	Cell proliferation / differentiation	When positive, indicates proliferative dysregulation associated with urothelial tumorigenesis and predictive of future recurrence potential.	Negative
ONECUT2 [8-9]	DNA methylation	Epithelial differentiation	When positive, indicates altered epithelial differentiation associated with urothelial tumorigenesis.	Negative
FGFR3 [11-14]	Somatic mutation	Growth factor receptor; MAPK pathway	When positive, indicates activating mutation typical of low-grade NMIBC and predictive of future recurrence potential.	Negative
HRAS [11,14]	Somatic mutation	RAS/MAPK oncogene	When positive, indicates RAS-pathway activation; complementary marker in multi-gene panels.	Negative
TERT [10, 14-15]	Somatic mutation	Telomerase promoter driver	**When positive, indicates early driver mutation detectable across stages and predictive of future recurrence potential.**	Positive

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Assay Description:

The AssureMDx test analyzes urothelial cells from urine using methylation-specific PCR to assess *TWIST1*, *OTX1*, and *ONECUT2*, and next-generation sequencing to detect mutations in *FGFR3*, *TERT*, and *HRAS*. These biomarker results are combined with hematuria type to generate a risk profile for urothelial carcinoma of the bladder and upper urinary tract. In clinical validation, the assay demonstrated 96% sensitivity, 73% specificity, 99% negative predictive value (NPV), and an AUC of 0.957 in the full hematuria cohort. Among patients with microscopic hematuria, performance remained robust with 93% sensitivity, 92% specificity, 99.7% NPV, and an AUC of 0.971—supporting its use as a non-invasive tool to triage lower-risk patients and potentially avoid unnecessary procedures.[1-4] Conversely, a positive test result indicates increased risk for urothelial carcinoma and may support the need for expedited urologic evaluation, particularly in patients who might otherwise be managed conservatively. A positive result is reported when one or more somatic mutations are detected, two or more methylation markers are hypermethylated, or one methylation marker is hypermethylated in the presence of gross hematuria. A negative result indicates all biomarkers are within normal parameters. These performance metrics confirm the ability of AssureMDx to provide clinically actionable information not otherwise available through cytology or imaging, supporting earlier and more accurate detection of urothelial carcinoma in patients presenting with hematuria.

Signature:

Shelly Gunn, MD, PhD, Laboratory Director
CLIA: 05D2197032; CAP Accredited: 9647012; CA License: CDF-90001843

Disclaimer: The AssureMDx test is regulated under the Clinical Laboratory Improvement Amendments (CLIA) of 1988 for high-complexity testing. The performing laboratory is accredited by the College of American Pathologists (CAP). This test has not been cleared or approved by the U.S. Food and Drug Administration. AssureMDx was developed and its performance characteristics were determined by Vesica Health. It is intended to assist clinicians in patient management and should be interpreted alongside other diagnostic findings and treatment guidelines. Clinical validation has been established only for this indication.

Limitations: The AssureMDx assay has been validated for use in patients aged 18 to 96, with an emphasis on those 50 years and older presenting with hematuria. The test's high accuracy, particularly in patients with microhematuria, supports its utility in reducing unnecessary invasive procedures in lower-risk cases. However, performance may vary in patients without hematuria, younger individuals, or those from diverse ethnic backgrounds. Results should be interpreted in the context of the patient's clinical history, symptoms, and additional diagnostic findings.

General information about AssureMDx can be found at www.VesicaHealth.com. If you have any questions regarding the report, please contact Vesica Health Client Services at customer.service@vesicahealth.com.

References: 1) de Jong JJ, et al. A Urine-based Genomic Assay Improves Risk Stratification for Patients with High-risk Hematuria Stratified According to the American Urological Association Guidelines. Eur Urol Oncol. 2023. 2) van Kessel KEM, et al. A Urine-Based Genomic Assay to Triage Patients with Hematuria for Cystoscopy. J Urol. 2020;204(3):482-490. 3) van Kessel KEM, et al. Validation of a DNA Methylation-Mutation Urine Assay to Select Patients with Hematuria for Cystoscopy. J Urol. 2017;197(6):1415-1422. 4) van Kessel KEM, et al. Evaluation of an Epigenetic Profile for the Detection of Bladder Cancer in Hematuria Patients. J Urol. 2016;195(3):601-607. 5) Renard I, et al. Identification and Validation of the Methylated TWIST1 and NID2 Genes through Real-Time Methylation-Specific Polymerase Chain Reaction Assays for the Noninvasive Detection of Primary Bladder Cancer in Urine Samples. Eur Urol. 2010;58(1):96-104. 6) Yegin GE et al. Hypermethylation of TWIST1 and NID2 in Tumor Tissues and Voided Urine in Urinary Bladder Cancer Patients. DNA Cell Biol. 2013;32(7):386-392. 7) Wan M, et al. Potential Role of TWIST1 and Its Methylation in Bladder Urothelial Carcinoma. Transl Cancer Res. 2024;13(11):6070-6086. 8) Kandimalla R, et al. Genome-Wide Analysis of CpG Island Methylation in Bladder Cancer Identifies Novel Biomarkers for Diagnosis and Prediction of Progression. Eur Urol Suppl. 2012;11(5):e328. 9) Beukers W, et al. The Use of Molecular Analyses in Voided Urine for the Assessment of Patients with Hematuria. PLoS One. 2013;8(10):e77657. 10) de Jong JJ, et al. A Molecular Urine Assay to Detect Recurrences During Surveillance of High-Risk Non-Muscle-Invasive Bladder Cancer. Bladder Cancer. 2024;10:233-242. 11) Kompier LC, et al. FGFR3, HRAS, KRAS, NRAS and PIK3CA Mutations in Bladder Cancer and Their Potential as Biomarkers for Surveillance and Therapy. PLoS One. 2010;5(12):e13821. 12) Zuiverloon T CM, et al. Fibroblast Growth Factor Receptor 3 Mutation Analysis on Voided Urine for Surveillance of Patients with Low-Grade Non-Muscle-Invasive Bladder Cancer. Clin Cancer Res. 2010;16(11):3011-3018. 13) van Kessel KEM, et al. FGFR3 Mutation Analysis in Voided Urine Samples to Decrease Cystoscopies and Cost in Non-Muscle-Invasive Bladder Cancer Surveillance: A Comparison of Three Strategies. J Urol. 2013;189(5):1676-1681. 14) de Jong FC, et al. A Genomic Urine Assay for Surveillance of Patients with Bladder Cancer Treated with Radiotherapy. Eur Urol Open Sci. 2024;62:131-139. 15) Allory Y, et al. Telomerase Reverse Transcriptase Promoter Mutations in Bladder Cancer: High Frequency Across Stages, Detection in Urine, and Lack of Association with Outcome. Eur Urol. 2014;65(2):360-366.

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Patient Test Result: Negative

A negative AssureMDx test result is associated with a very low likelihood of urothelial carcinoma. In clinical validation, the post-test cancer risk ranged from <1% to 2%, representing a greater than 10-fold reduction in risk compared to baseline across hematuria subgroups.[1-2]

Clinical Interpretation

AssureMDx evaluates six validated molecular biomarkers to identify DNA alterations associated with urothelial carcinoma. The assay integrates these biomarker results with hematuria type to generate a patient-specific molecular risk profile for bladder and upper tract urothelial carcinoma. Performance has been validated in prospective multi-center cohorts demonstrating high sensitivity and negative predictive value for urothelial carcinoma detection.[1–4]

A ***negative test result*** indicates all biomarkers are within normal parameters and supports a very low likelihood of malignancy. A positive test result indicates the presence of one or more molecular alterations associated with tumor development or progression and should be interpreted as an increased likelihood of urothelial carcinoma. These findings are intended to assist clinicians in determining the appropriate timing and need for urologic evaluation based on each patient's presentation and risk factors and may support conservative management in appropriate patients, particularly those at lower clinical risk.

Biomarker Status:

Biomarker	Category	Functional Role	Clinical Relevance	Result
TWIST1 [5-7]	DNA methylation	EMT / invasion regulator	When positive, indicates aggressive tumor biology with promoter hypermethylation significantly associated with progression, stage, grade, and recurrence potential.	Negative
OTX1 [8-10]	DNA methylation	Cell proliferation / differentiation	When positive, indicates proliferative dysregulation associated with urothelial tumorigenesis and predictive of future recurrence potential.	Negative
ONECUT2 [8-9]	DNA methylation	Epithelial differentiation	When positive, indicates altered epithelial differentiation associated with urothelial tumorigenesis.	Negative
FGFR3 [11-14]	Somatic mutation	Growth factor receptor; MAPK pathway	When positive, indicates activating mutation typical of low-grade NMIBC and predictive of future recurrence potential.	Negative
HRAS [11,14]	Somatic mutation	RAS/MAPK oncogene	When positive, indicates RAS-pathway activation; complementary marker in multi-gene panels.	Negative
TERT [10, 14-15]	Somatic mutation	Telomerase promoter driver	When positive, indicates early driver mutation detectable across stages and predictive of future recurrence potential.	Negative

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Assay Description:

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Signature:

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Limitations: The AssureMDx assay has been validated for use in patients aged 18 to 96, with an emphasis on those 50 years and older presenting with hematuria. The test's high accuracy, particularly in patients with microhematuria, supports its utility in reducing unnecessary invasive procedures in lower-risk cases. However, performance may vary in patients without hematuria, younger individuals, or those from diverse ethnic backgrounds. Results should be interpreted in the context of the patient's clinical history, symptoms, and additional diagnostic findings.

General information about AssureMDx can be found at www.VesicaHealth.com. If you have any questions regarding the report, please contact Vesica Health Client Services at customer.service@vesicahealth.com.

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Patient Test Result: Test Not Performed (TNP – Sample did not meet acceptance criteria)

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Patient Test Result: Test Not Performed

The AssureMDx test was not performed for this patient. One or more of the following criteria were not met: (1) clinical eligibility requirements based on information provided; (2) pre-analytic specimen integrity criteria (e.g., expired collection timeframe or improper handling); or (3) sufficient DNA quantity or quality for molecular analysis. Please contact Vesica Health Client Services at customer.service@vesicahealth.com for questions about specimen suitability or to discuss recollection if clinically appropriate.

Assay Description:

Note: This test was not performed; the following assay description is for informational purposes only and should not be used for clinical interpretation.

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