

# GALEAS® Bladder

A non-invasive, NGS-based test for identifying bladder cancer from a urine sample

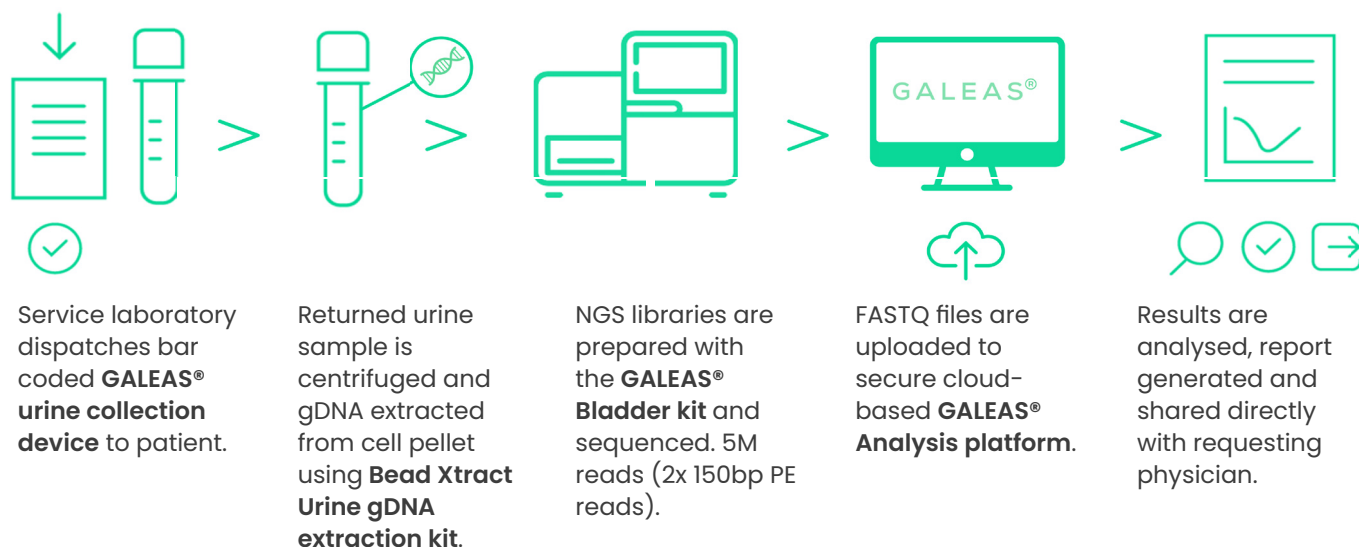
## Introduction

Despite intensive research into biomarkers for the non-invasive diagnosis of urothelial bladder cancer (UBC), the mainstay of detection remains flexible cystoscopy. Commercial urine tests exist; however, none have been widely accepted into routine clinical practice due to poor performance and/or poor evidence.

The majority of current tests are based on RNA or protein expression levels, however as these are often not unique to UBC or causally linked to the disease, they tend to lack specificity and often lack sensitivity to detect small or low-grade tumors. The ideal non-invasive test should detect all UBCs whilst not generating false-positive results from non-malignant urological conditions.

GALEAS® Bladder is a molecular biomarker test, developed in partnership with researchers at the University of Birmingham, (UK), that leverages targeted Next Generation Sequencing (NGS) to interrogate the key somatic mutations found across all grades and stages of bladder cancer. Based on ultra-sensitive targeted NGS chemistry developed by Nonacus, the GALEAS® Bladder kit enables the highly sensitive and accurate detection of tumor-derived DNA in a urine sample, offering a viable molecular biomarker alternative to flexible cystoscopy.

## GALEAS® Bladder workflow



From urine sample to test result, the **GALEAS® Bladder test** consists of four components validated and optimised as a complete workflow to deliver uncompromising sensitivity and specificity:

### GALEAS® Urine Collection Device

The urine collection device is shipped ready for dispatch direct to patients. It contains a simple cardboard collection device which is easy to use (ensuring good sample taking and return rates) and flat packs reducing storage and shipping costs. The sample is returned to the lab in a 50ml barcoded Falcon tube containing a proprietary preserver solution, stabilising urine samples for up to 28 days at room temperature (18 to 25°C). The collection kit has been validated with our DNA extraction kit and NGS library preparation reagents to ensure optimal performance. Furthermore, the tube is barcoded to provide full traceability.

### Bead Xtract gDNA Extraction Kit

Our genomic DNA extraction kit is based on a simple, magnetic bead-based protocol making it flexible and scalable for laboratories. Using 50 ml of urine, samples are centrifuged and genomic DNA (gDNA) extracted from the cell pellet. The quick and easy workflow (Figure 1) supports manual or automated preparation of 1 – 96 samples in a single batch. Extraction performance has been proven to be highly reproducible from batch to batch (Figure 2).

### Bead Xtract workflow

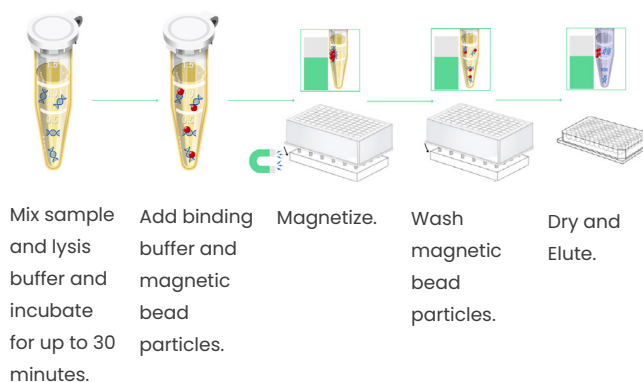


Figure 1: Bead Xtract Urine gDNA kit workflow

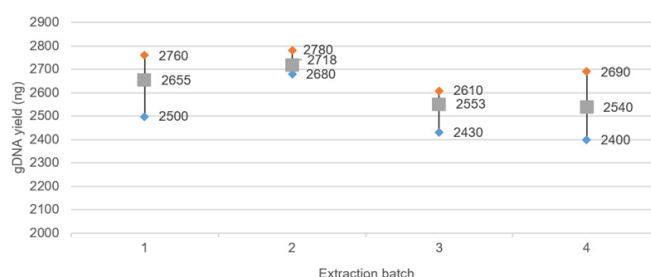


Figure 2: gDNA yield (ng) from pooled urine pellets across 4 extraction batches using Bead Xtract Urine gDNA extraction kit. The CV% between batches was 3.2%.

GALEAS® Bladder Kit

The GALEAS® Bladder kit uses ultra-sensitive NGS chemistry to target promoter and exonic regions of 23 of the most relevant genes associated with bladder cancer (Table 1). The somatic mutations covered by the kit have been shown to detect 96% of bladder cancers in over 664 clinical samples <sup>(1,2)</sup>.

AKT1	ERBB2	NRAS
BRAF	ERBB3	PIK3CA
C3orf70	ERCC2	RHOB
CDKN1A	FBXW7	RXRA
CDKN2A	FGFR3	SF3B1
CREBBP	HRAS	TERT (promoter)
CTNNB1	KDM6A	TP53
ELF3	KRAS	

Table 1: Gene list for GALEAS® Bladder. Variants in these genes covered 96% of all stages and grades of bladder cancer cases.

The targeted sequencing panel is 18.49 kB in size, requiring 1.5 Gb (approximately 5M, 2x150pb paired end reads) of sequence per sample to provide raw coverage of 30,000x and a mean UMI collapsed coverage of 2500x. Libraries generated by the kit are compatible with all Illumina sequencers and 384 patient/sample indexes ensure that a laboratory can use the GALEAS® Bladder kit on the smallest to the largest output sequencers. High on-target rates and excellent uniformity of coverage deliver efficient sequencing ensuring the test can be delivered without incurring unnecessary sequencing costs.

GALEAS® Analysis Software

Our cloud-based bioinformatic analysis solution for GALEAS® Bladder is included in the cost of the panel and has been purpose built to allow rapid analysis and reporting. Providing a simple, clear and concise answer, the GALEAS® Bladder report is easy to interpret and requires no specialist knowledge. Analysis with the GALEAS® analysis software is easily scalable, with no minimum sample numbers and bulk uploads of sequencing data (FASTQ files), allowing from 1 to 384 samples to be analysed at any one time.

GALEAS® Bladder Technical Performance

Performance of the GALEAS® Bladder kit was assessed in a cohort of reference samples containing mutations across the spectrum of the GALEAS® Bladder panel. GALEAS® Bladder shoes high sensitivity, detecting over 95% of variants with a variant allele frequency (VAF) greater than 0.1%.

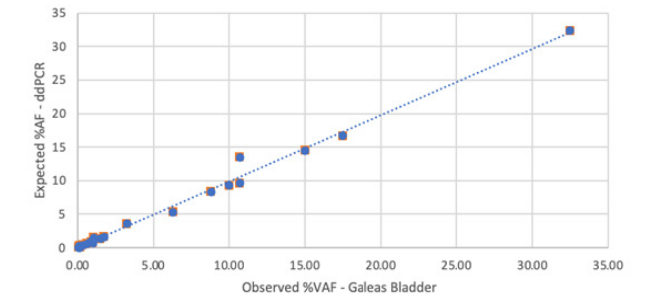


Figure 3: GALEAS® Bladder shows high sensitivity detecting VAFs down to 0.1%.

GALEAS® Bladder Clinical Validation

GALEAS® Bladder performance was assessed on 664 urine samples from three UK clinical cohorts, showing high detection sensitivity and specificity across all stages and grades of bladder cancer (Table 3).

	Sensitivity	Specificity
pTa	87%	85%
T1	95%	85%
T2+	86%	85%
G1	78%	85%
G2	91%	85%
G3	91%	85%
NMIBC	90%	85%
MIBC	86%	85%

Table 3: Sensitivity and Specificity of GALEAS® Bladder across all stages and grades of bladder cancer.

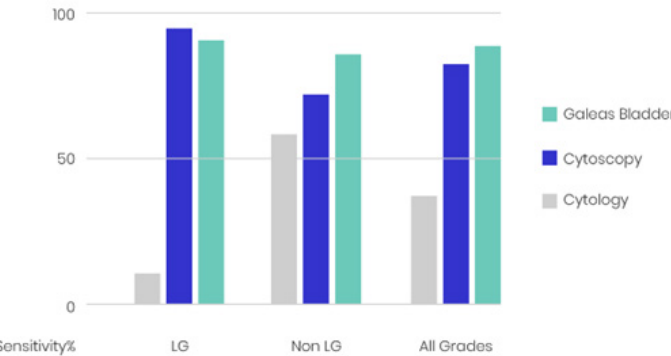


Figure 4: GALEAS® Bladder performance compared to cystoscopy and cytology across all stages of bladder cancer.

## Summary

GALEAS® Bladder provides a non-invasive, sample to report, molecular triage for patients with hematuria. It provides a highly sensitive test that can quickly and accurately rule in or out the likely presence of bladder cancer. Validated in over 600 patient urine samples, the test has been shown to perform comparably across all stages and grades of bladder cancer.

### Learn more

To learn more about GALEAS® Bladder and other GALEAS products, and to download protocols, application notes and white papers please visit [www.nonacus.com](http://www.nonacus.com).

### References

1. Douglas G. Ward, et al, Highly Sensitive and Specific Detection of Bladder Cancer via Targeted Ultra-deep Sequencing of Urinary DNA, European Urology Oncology, 2022, ISSN 2588-9311, <https://doi.org/10.1016/j.euo.2022.03.005>.
2. Ward DG, et al, Targeted deep sequencing of urothelial bladder cancers and associated urinary DNA: a 23-gene panel with utility for non-invasive diagnosis and risk stratification. BJU Int. 2019 Sep;124(3):532-544. <https://doi.org/10.1111/bju.14808> Epub 2019 Jun 19. PMID: 31077629; PMCID: PMC6772022.

Ordering information	Pack size	Catalogue number
GALEAS® Urine collection device	1	85%
GALEAS® Bead Xtract: Urine gDNA extraction kit	96 samples	85%
GALEAS® Bladder kit (includes 1-96 indexes and complimentary analysis of FASTQ files using GALEAS® Analysis software for up to 96 samples)	96 samples	85%
GALEAS® Bladder kit (includes 97-192 indexes and complimentary analysis of FASTQ files using GALEAS® Analysis software for up to 96 samples)	96 samples	85%
GALEAS® Bladder kit (includes 193-288 indexes and complimentary analysis of FASTQ files using GALEAS® Analysis software for up to 96 samples)	96 samples	85%
GALEAS® Bladder kit (includes 289-384 indexes and complimentary analysis of FASTQ files using GALEAS® Analysis software for up to 96 samples).	96 samples	85%
GALEAS® Bladder validation control kit	48	85%

### Nonacus Limited

Quinton Business Park  
11 Ridgeway  
Birmingham  
B32 1AF

[info@nonacus.com](mailto:info@nonacus.com)

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