Figure 3. RT-qPCR assay of GS_D2 and GS_D10 signatures in the validation cohort (n=525 samples). NMIBC, Non-muscle invasive bladder cancer; MIBC, Muscle invasive bladder cancer.

Prospective, blinded, international and multicenter validation of a gene expression test for the non-invasive diagnosis of bladder cancer

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OBJECTIVE

Current standard methods used to detect and monitor bladder cancer (BC) are invasive or have low sensitivity. We have previously reported four non-invasive tests for BC diagnosis based on the gene expression patterns of urine.[1,2] Here, we present the clinical validation results of our tests in independent European and Asian cohorts.

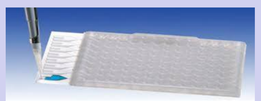
METHODS

- Consecutive voided urine samples from BC patients and controls were collected in five European (N=789) and one Asian (N=93) centre. Finally, 525 samples from the European trial and 69 from the Asian study were successfully analyzed.

Table 1. Pathological features of tumour samples included in each participating centre in the European and Asiatic cohorts .

	European cohort		Asiatic cohort	
	Patients	Controls	Patients	Controls
n male	171	159	28	28
n female	45	150	10	3
male/female	3,8	1,1	2,8	10,7
average age	70	55	63,6	63,9
NMIBC LG	88	-	6	-
NMIBC HG	90	-	9	-
MIBC	38	-	23	-
TOTAL	**216**	**309**	**38**	**31**

- Gene expression values were quantified using TaqMan Arrays. The same cut-off as previously reported for discrimination between tumour and controls was used in this study. [1,2]
- Results from the most accurate gene signature were correlated to clinical parameters, such as cytology results, tumour multiplicity and tumour size using ANOVA test.



[1] Mengual L et al: **Validation study of a noninvasive urine test for diagnosis and prognosis assessment of bladder cancer: evidence for improved models.** *J Urol* 2014, **191**:261-269.
[2] Mengual L et al: **Gene expression signature in urine for diagnosing and assessing aggressiveness of bladder urothelial carcinoma.** *Clin Cancer Res* 2010, **16**:2624-2633.

Table 2. Gene expression signatures for BC diagnosis

Gene symbol	GS_D2	GS_D5	GS_D10	GS_D12
IGF2	■	■	■	■
MAGEA3	■	■	■	■
KLF9		■	■	■
CRH		■	■	■
SLC1A6		■	■	■
POSTN			■	■
EBF1			■	
CFH			■	
MCM10			■	
MMP12			■	
TERT				■
AHNAK2				■
ANXA10				■
CTSE				■
KRT20				■
PPP1R14D				■

RESULTS

Figure 1. Diagnostic performance of the four gene expression models in the European (a and b) and Asiatic (c) cohorts.

a)

Gene models	GS_D2	GS_D5	GS_D10	GS_D12
SN (%)	81.48	79.17	79.63	78.70
SP (%)	91.26	91.59	93.53	93.20
PPV (%)	86.70	86.80	89.58	89.01
NPV (%)	87.58	86.28	86.79	86.23
ER (%)	12.76	13.52	12.19	12.76

c)

Gene models	GS_D2	GS_D5	GS_D10	GS_D12
SN (%)	73.68	84.21	84.21	84.21
SP (%)	80.65	83.87	87.10	90.32
PPV (%)	82.35	86.46	88.89	91.43
NPV (%)	71.43	81.25	81.82	82.35
ER (%)	23.19	15.94	14.49	13.04

b)

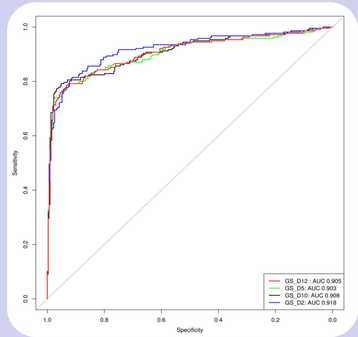
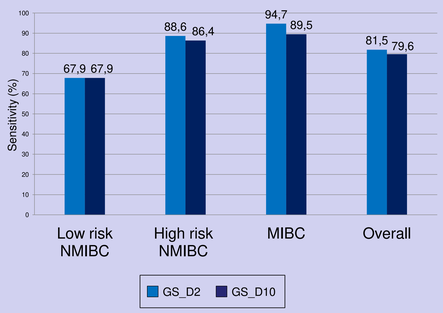
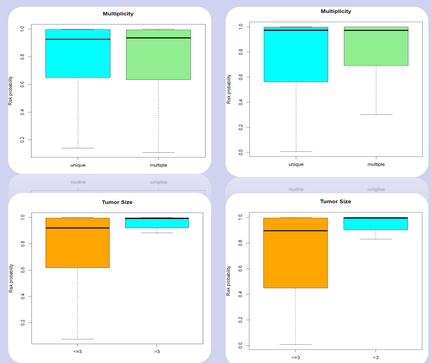


Figure 2. Diagnostic performance of the GS_D2 and GS_10 models in the European cohort

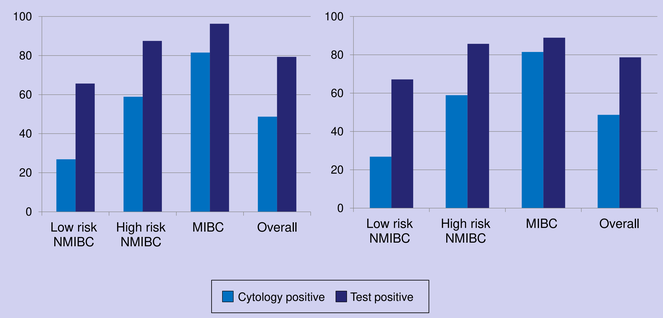
a) Diagnostic performance of GS_D2 and GS_10 in the different bladder cancer risk groups



b) Correlation between the performance of GS_D2 and GS_D10 with tumour multiplicity and size.



c) Cytology and GS_D2 and GS_10 tests sensitivity based on risk groups on tumour samples



Abbreviations: *N = number; SN = sensitivity; SP = specificity; PPV = positive predictive value; NPV = negative predictive value; ER = overall error rate; AUC = Area Under Curve. NMIBC = Non-muscle invasive bladder cancer; MIBC = Muscle invasive bladder cancer, LG=Low-grade, HG=High grade.*

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CONCLUSIONS

Our study proves that our non-invasive diagnostic BC tests can be **reproduced in independent cohorts and in an external laboratory**. All the four gene expression signatures have shown **equal or superior performance to the current gold standard** in the present and previously reported validation studies. Consequently, they may be taken for consideration as a **molecular test applicable to clinical practice in the management of BC**.