

MCM5 ELISA; A Sensitive and Specific Diagnostic Test for Bladder Cancer

Introduction

Bladder cancer is the 5th most common cancer in Europe with over 123,000 new cases every year and 40,000 deaths (1). The gold standard test for bladder tumour detection in patients presenting with haematuria and/or lower urinary tract symptoms with a suspicion of malignancy, is cystoscopy. However this is an invasive uncomfortable and relatively expensive procedure, carrying the risk of infection (up to 2% of cases contract UTI (2)). In addition, poor visualisation, as a result of inflammatory conditions, bleeding and/or flat urothelial lesions can lead to up to 30% of tumours being missed (3). Due to the high recurrence rate of bladder cancer, the costs of surveillance of patients (regular cystoscopies) bladder cancer has the highest lifetime costs of any cancer. The ability to detect bladder cancer, and recurrence of bladder cancer through the use of a non-invasive highly sensitive and specific urinary marker could improve management of the disease by decreasing the number of cystoscopies, and potentially allowing earlier detection of tumour recurrence.

MCM5 is an excellent urinary biomarker for bladder cancer. Expression of MCM5 proteins (an essential component for DNA replication) is an indicator of proliferation. Proliferating cells express MCM5 protein at a high level, whilst quiescent terminally differentiated cells, and senescent cells lack MCM5 expression. In normal urothelium MCM5 expression is restricted to the basal stem-transit proliferative compartment. In contrast, both non-invasive and invasive urothelial carcinomas express MCM5 in all layers of the urothelium including superficial epithelial cells. This results in the exfoliation of MCM5-expressing tumour cells into the urine. Therefore the presence of MCM5 in the urine sediment is an excellent indicator of the presence of a urological tumour.

Stoeber et al (4) demonstrated that immunofluorometric detection of MCM5 in urine sediment is a highly sensitive and specific diagnostic test for bladder cancer, with a sensitivity of 92% and specificity of 78%. However, this method of detection is complex and the “Arquer Diagnostics MCM5 ELISA” represents a significant improvement in the usability of the test.

Methods:

Voided urine specimens were obtained from 510 patients, presenting with haematuria and/or lower urinary tract symptoms at 3 leading UK hospitals. All patients underwent flexible cystoscopy following production of the urine sample. Patients unable to provide 30mL of urine and those subsequently diagnosed with calculi or with renal or prostate malignancies were excluded from analysis.

Urine samples were processed per the MCM5 ELISA instructions for use.

1. **Creating a cell pellet from the urine sample;** 30mL of urine was centrifuged at 1,500g for 5 minutes, supernatant urine was decanted off.
2. **Lysing the cells to release the MCM5 protein (if present);** the urine sediment was re-suspended in 300ul of a proprietary lysis buffer. The lysates were then frozen at -20°C
3. **Sample Analysis,** Samples were analysed using a 96 well microplate manual ELISA in a blinded fashion.

Patient data was collected and cystoscopy, imaging and biopsy results were compared to the MCM5 ELISA result.

Of the 510 patients recruited 164 were eliminated (36 calculi, 23 prostate cancer, 5 renal cancers, 2 withdrawn consent and 98 no clinical information available/technical issue). Out of the 346 eligible samples 29 were confirmed as having bladder cancer and 317 were negative.

Overall Sensitivity of 83% and Specificity 77%

Arquer's MCM5 ELISA demonstrated an overall sensitivity of 83%, with 24 out of 29 cancer positive cases being diagnosed correctly. Overall specificity was 77% with 244 out of 317 cancer free patients being correctly diagnosed.

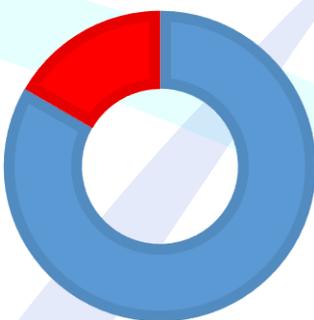
98% Negative Predictive Value

The negative predictive value of the Arquer MCM5 ELISA test is 98% (based on a prevalence of 8.38%).

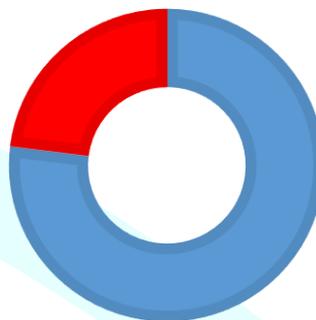
		MCM5 ELISA		
		Positive	Negative	Total
Bladder Cancer	Positive	24	5	29
	Negative	73	244	317
	Total	97	249	346

Table 1: Clinical data comparison with MCM5 result.

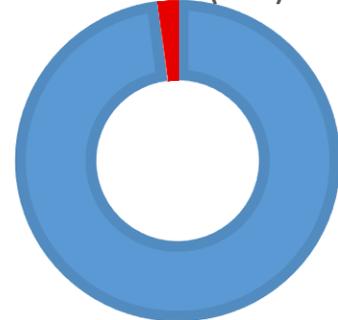
SENSITIVITY (83%)



SPECIFICITY (77%)



NEGATIVE PREDICTIVE VALUE (98%)



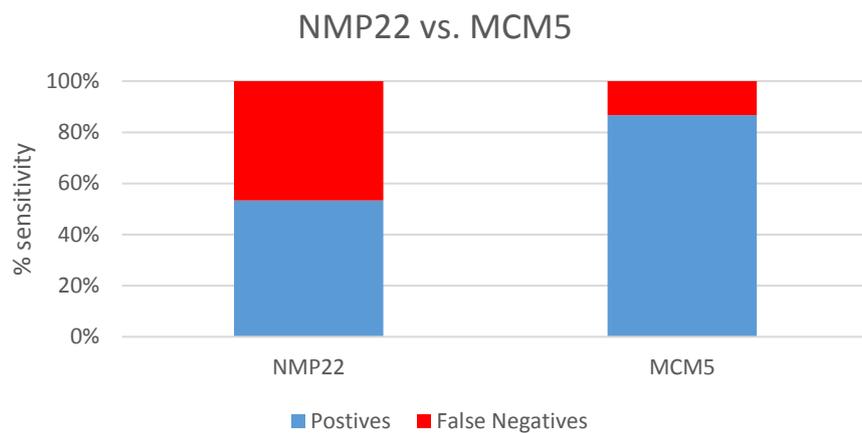
More Sensitive than Current Biomarker Tests

NMP22 ELISA

100 samples were tested in parallel using urine based ELISA tests for NMP22 and MCM5. In this sub-set of patients NMP22 demonstrated a sensitivity of 53.3% (16/30), whilst MCM5 showed a sensitivity of 86.7% (26/30). All 16 of the patients testing positive for NMP22 also tested positive for MCM5.

	Patient total	Tested Positives	Confirmed Positives	Sensitivity
NMP22	100	16	30	53.3%
MCM5	100	26	30	86.7%

Table 2: NMP22 comparison to MCM5

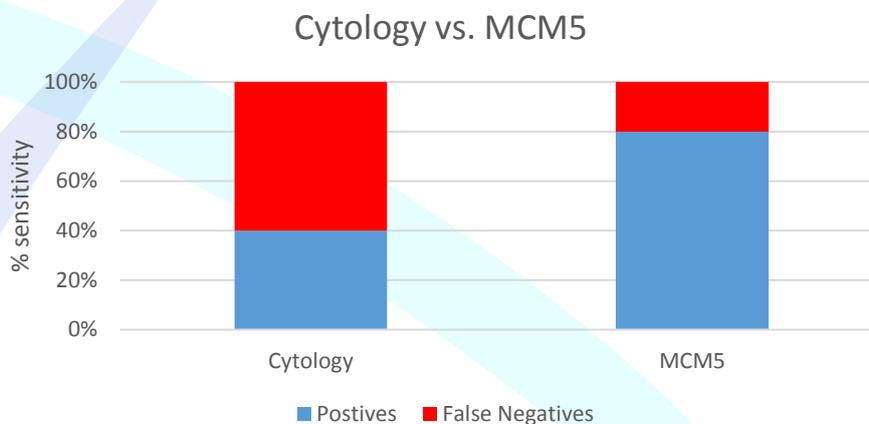


Urine Cytology

98 patient samples were tested by urine cytology and MCM5 ELISA. Of 10 cancer positive samples, Arquer's MCM5 ELISA test detected 8. The 2 missed cases were a G1pTa (low grade cancer) and a cancer of unknown grade. In these 2 cases the cytology also returned a negative result of normal and equivocal. Cytology detected 4 out of 10 cancers, with equivocal or atypical results in a further 4 cancers.

	Patient total	Tested Positives	Confirmed Positives	Sensitivity
Cytology	98	4	10	40%
MCM5	98	8	10	80%

Table 3: Cytology comparison to MCM5



MCM5 May be Indicative of a Urogenital Cancer

On further investigation of the samples removed from the study, a number of haematuria patients were subsequently diagnosed with a urogenital malignancy (Prostate, Renal or Testicular Cancer). This could suggest that a positive MCM5 result may be indicative of other malignancies within the urogenital tract and may act as a prompt for the clinician to perform further investigation.

<u>Patient ID</u>	<u>Negative tests</u>	<u>Diagnosis</u>	<u>MCM5 concentration (pg/ml)</u>
2047	Cytology-refused cystoscopy	Bladder Cancer G1/G2pTa TCC*	250.2
2091	Cystoscopy, Scans	Testicular Cancer	36.0
2020	-	Prostate Cancer G3+3	28.8
2035	-	Prostate Cancer G4+4	121.6
2087	-	Prostate Cancer G4+5	71.2
2105	-	Prostate Cancer G3+3	41.2
2204	-	Renal Cell Carcinoma Papillary pT1bNoMo	36.3
2239	-	Renal Cell Carcinoma Papillary pT1aNxMx	436.4
2232	-	Prostate Cancer G4+3 T2b	24.5

Table 4: Investigation into MCM5 positive cases excluded from the study.

*This patient represented 5 months after the positive MCM5 result/refused cystoscopy with bladder cancer

Conclusion

We have demonstrated that the Arquer Diagnostics MCM5 ELISA test is a highly sensitive and specific test for bladder cancer with a high negative predictive value. Comparisons to alternative urine tests demonstrate that the Arquer MCM5 ELISA has superior sensitivity to both NMP22 and urine cytology, with the MCM5 ELISA identifying all of the cases detected by these urine based methods.

References

1. **Eur J Cancer.** 2013 Apr;49(6):1374-403
2. **J Urol.** 2015 Feb;193(2):548-51.
3. **Urology** 57: 690–694.
4. **J Natl Cancer Inst.** 2002 Jul 17;94(14):1071-9.