Resurrecting the role of urine cytology in bladder cancer surveillance

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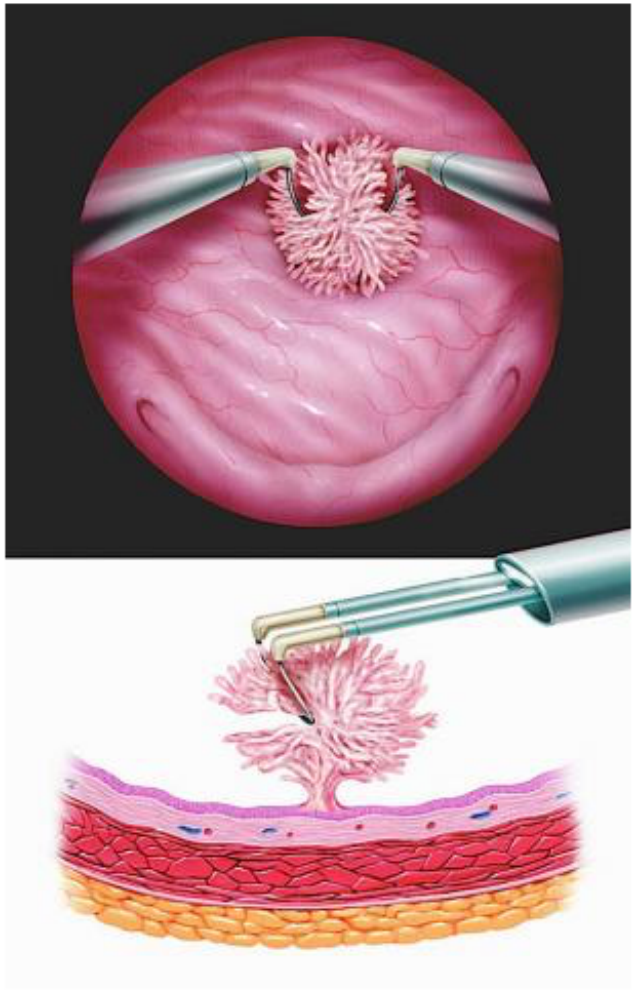
Bladder cancer

- 9th most common cancer worldwide
- 2 distinct disease entities

  - Non-muscle invasive disease (70%)
    - Transurethral resection of bladder tumour (TURBT)
    - Intravesical chemotherapy or BCG

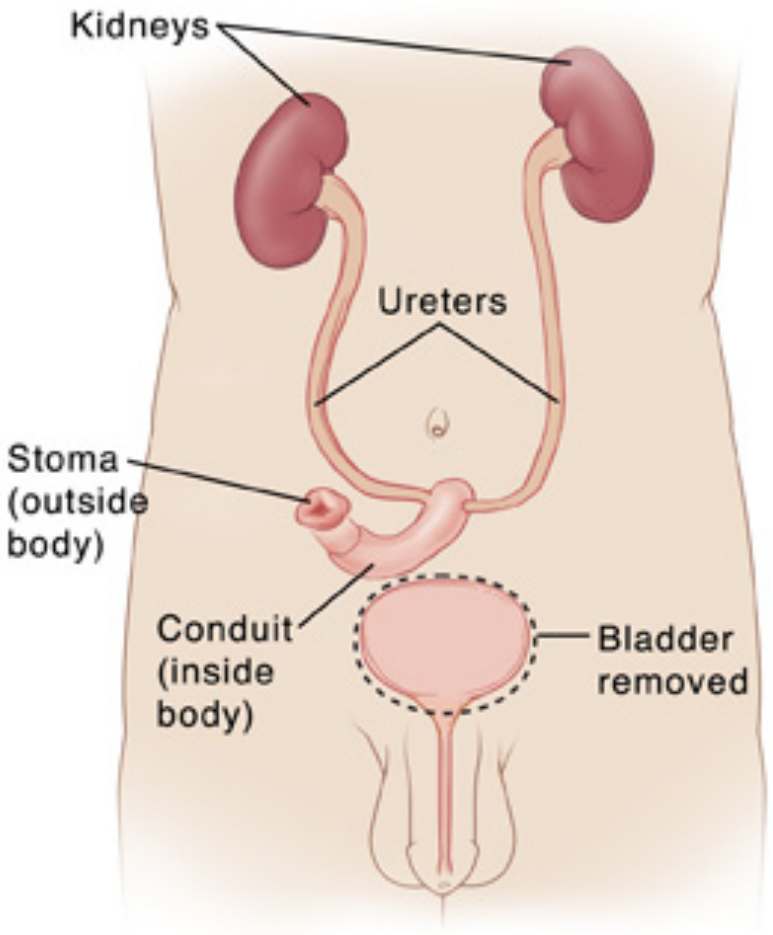
Management of NMIBC

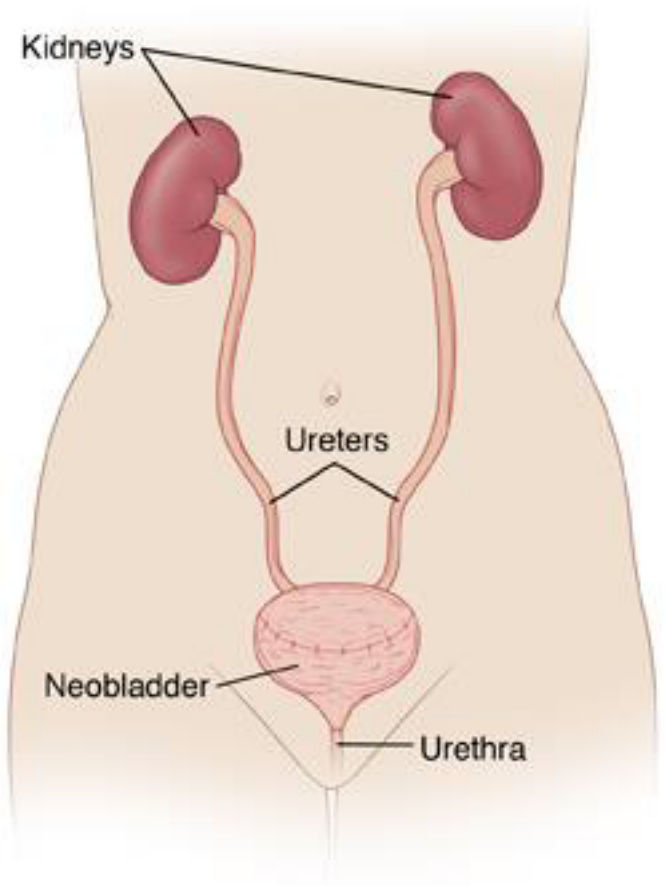


Bladder cancer

- 9th most common cancer worldwide
- 2 distinct disease entities
  - Non-muscle invasive disease (NMIBC) (70%)
    - Transurethral resection of bladder tumour (TURBT)
    - Intravesical chemotherapy or BCG
  - Muscle invasive disease (MIBC) (30%)
    - Radical cystectomy
    - Adjuvant chemotherapy or chemoradiotherapy

Management of MIBC





The burden of care

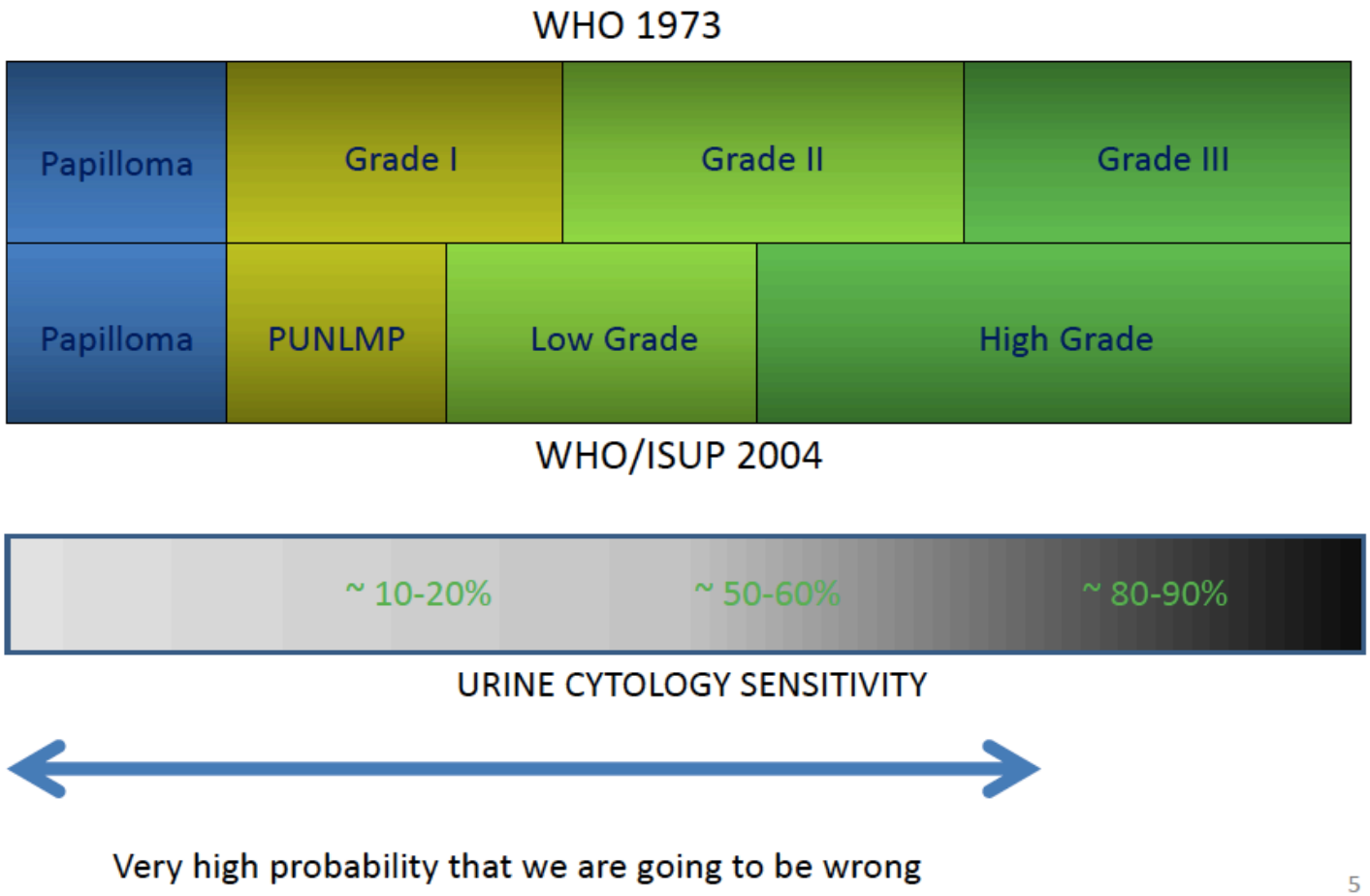
- Bladder cancer is the 6th leading cause of cancer in the EU
- Bladder cancer is among the most costly for healthcare
  - In 2012, expenditure in EU totaled 4.9 billion Euros
- Life long monitoring of patients with NMIBC
- For high grade tumours
  - Cystoscopy 3 monthly for 2 years
  - Cystoscopy 6 monthly for 5 years
  - Annually thereafter
  - High rate of recurrence (up to 70% within 5 years)
- Impacts negatively on the access to diagnostic cystoscopy

Access to cystoscopy (Nov 2018)

Diagnostic Cystoscopy	NHS Scotland		Surveillance Cystoscopy	NHS Scotland
Number on List	3073		Number on List	5845
Number waiting >4 weeks	1255		Beyond due date	1371
Number waiting >6 weeks	860		>3 months overdue	788
% waiting <6 weeks	72%		>5 months overdue	341

scottish access collaborative

The problem with urine cytology



The role of urine cytology in surveillance

- Routine use has been abandoned in many units
- The sensitivity of cytology for even high grade disease has diminished [1]

- Potential explanations:
  - Reporting is highly subjective and dependent upon experience of cytopathologist
  - No consensus on optimal urine collection or preparation

1. Halling KC, King W, Sokolova IA, Meyer RG, Burkhardt HM, Halling AC, et al. A comparison of cytology and fluorescence in situ hybridization for the detection of urothelial carcinoma. J Urol. 2000 Nov. 164(5):1768-75.

Re-inventing urine cytology

- Central project to my NRS Fellowship award
  - Commercial partner Cytosystems Ltd
  - Academic partners in St Andrews University
  - Funded by Innovate UK and Horizon 2020 grants

- Clinical cohort
  - 970 patients recruited via urology one stop clinic

- Optimising specimen handling
  - All samples centrifuged and fixed within 1hr voiding

- Optimising cytology reporting
  - A small panel of experienced uro-pathologists reporting fixed samples

  - Automated image analysis of prepared slide
    - Morphometric cellular characteristics captured
    - Immunocytochemical staining with a biomarker of cell proliferation (MCM-2)

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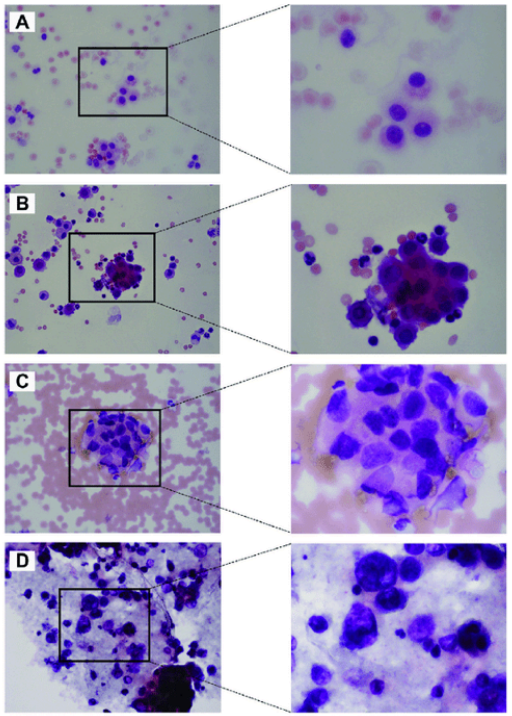
Development of automated image analysis cytology platform

- Cyto-histology reporting



Benign

Malignant

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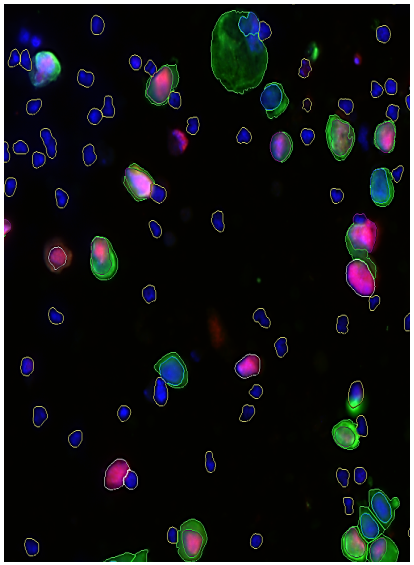
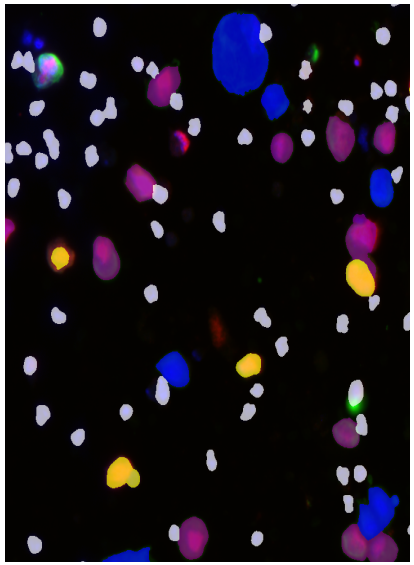
Development of automated image analysis cytology platform

- Image analysis of IF labelled biomarker (MCM-2)

Nuclear and cell segmentation



Biomarker based cell classification



Captured Data:
Morphometric parameters of every individual nucleus and cell e.g. size, shape, texture

Co-registered with IF labelled biomarker of cell proliferation

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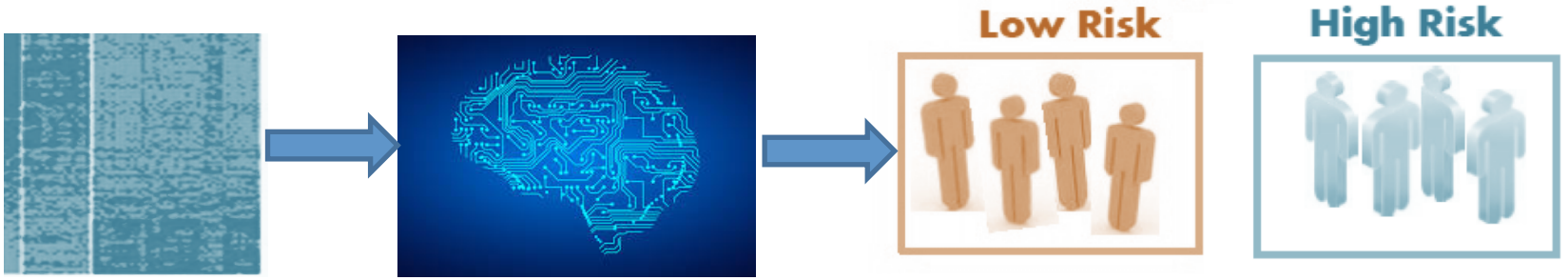
Development of automated image analysis cytology platform

- Software algorithm for image analysis



Raw clinical and image capture data

Machine Learning Using Decision Tree analysis and Artificial neural networks

Prediction made on the risk of cancer recurrence

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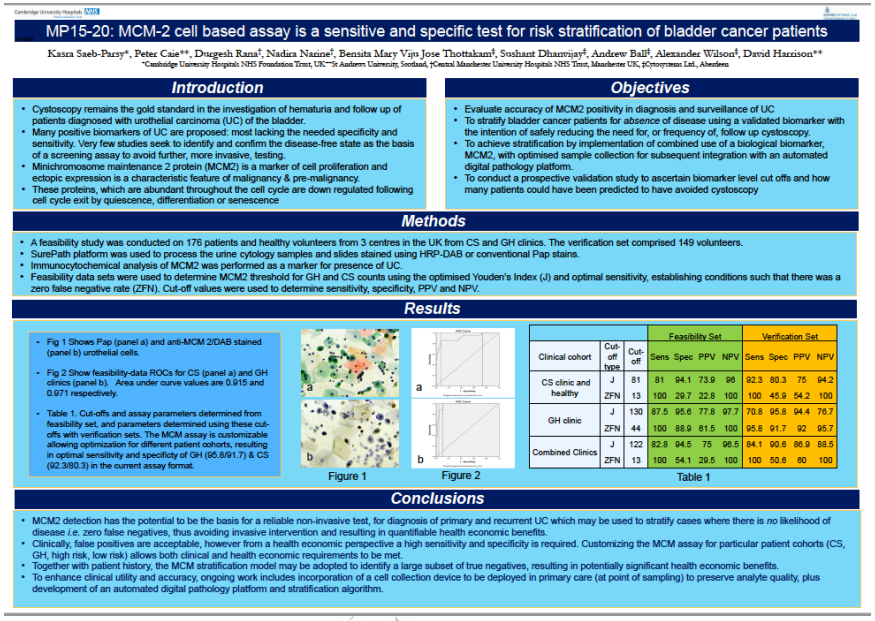
Development of automated image analysis cytology platform

- Iterative process to train the system to identify malignant cell types
- Sensitivity and specificity of the test approached 95%



MP15-20: MCM-2 cell based assay is a sensitive and specific test for risk stratification of bladder cancer patients

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Introduction

- Cystoscopy remains the gold standard in the investigation of hematuria and follow up of patients diagnosed with urothelial carcinoma (UC) of the bladder.
- Many positive biomarkers of UC are proposed: most lacking the needed specificity and sensitivity. Very few studies seek to identify and confirm the disease-free state as the basis of a screening assay to avoid further, more invasive, testing.
- Minichromosome maintenance 2 protein (MCM2) is a marker of cell proliferation and ectopic expression is a characteristic feature of malignancy & pre-malignancy.
- These proteins, which are abundant throughout the cell cycle are down regulated following cell cycle exit by quiescence, differentiation or senescence

Objectives

- Evaluate accuracy of MCM2 positivity in diagnosis and surveillance of UC
- To stratify bladder cancer patients for *absence* of disease using a validated biomarker with the intention of safely reducing the need for, or frequency of, follow up cystoscopy.
- To achieve stratification by implementation of combined use of a biological biomarker, MCM2, with optimised sample collection for subsequent integration with an automated digital pathology platform.
- To conduct a prospective validation study to ascertain biomarker level cut offs and how many patients could have been predicted to have avoided cystoscopy

Methods

- A feasibility study was conducted on 176 patients and healthy volunteers from 3 centres in the UK from CS and GH clinics. The verification set comprised 149 volunteers.
- SurePath platform was used to process the urine cytology samples and slides stained using HRP-DAB or conventional Pap stains.
- Immunocytochemical analysis of MCM2 was performed as a marker for presence of UC.
- Feasibility data sets were used to determine MCM2 threshold for GH and CS counts using the optimised Youden's Index (J) and optimal sensitivity, establishing conditions such that there was a zero false negative rate (ZFN). Cut-off values were used to determine sensitivity, specificity, PPV and NPV.

Results

- Fig 1 Shows Pap (panel a) and anti-MCM 2/DAB stained (panel b) urothelial cells.
- Fig 2 Show feasibility-data ROCs for CS (panel a) and GH clinics (panel b). Area under curve values are 0.915 and 0.971 respectively.
- Table 1. Cut-offs and assay parameters determined from feasibility set, and parameters determined using these cut-offs with verification sets. The MCM assay is customizable allowing optimization for different patient cohorts, resulting in optimal sensitivity and specificty of GH (95.8/91.7) & CS (92.3/80.3) in the current assay format.

Figure 1

Figure 2

			Feasibility Set				Verification Set			
Clinical cohort	Cut-off type	Cut-off	Sens	Spec	PPV	NPV	Sens	Spec	PPV	NPV
CS clinic and healthy	J	81	81	94.1	73.9	96	92.3	80.3	75	94.2
	ZFN	13	100	29.7	22.8	100	100	45.9	54.2	100
GH clinic	J	130	87.5	95.6	77.8	97.7	70.8	95.8	94.4	76.7
	ZFN	44	100	88.9	61.5	100	95.8	91.7	92	95.7
Combined Clinics	J	122	82.8	94.5	75	96.5	84.1	90.6	86.9	88.5
	ZFN	13	100	54.1	29.5	100	100	50.6	60	100

Table 1

Conclusions

- MCM2 detection has the potential to be the basis for a reliable non-invasive test, for diagnosis of primary and recurrent UC which may be used to stratify cases where there is *no* likelihood of disease *i.e.* zero false negatives, thus avoiding invasive intervention and resulting in quantifiable health economic benefits.
- Clinically, false positives are acceptable, however from a health economic perspective a high sensitivity and specificity is required. Customizing the MCM assay for particular patient cohorts (CS, GH, high risk, low risk) allows both clinical and health economic requirements to be met.
- Together with patient history, the MCM stratification model may be adopted to identify a large subset of true negatives, resulting in potentially significant health economic benefits.
- To enhance clinical utility and accuracy, ongoing work includes incorporation of a cell collection device to be deployed in primary care (at point of sampling) to preserve analyte quality, plus development of an automated digital pathology platform and stratification algorithm.

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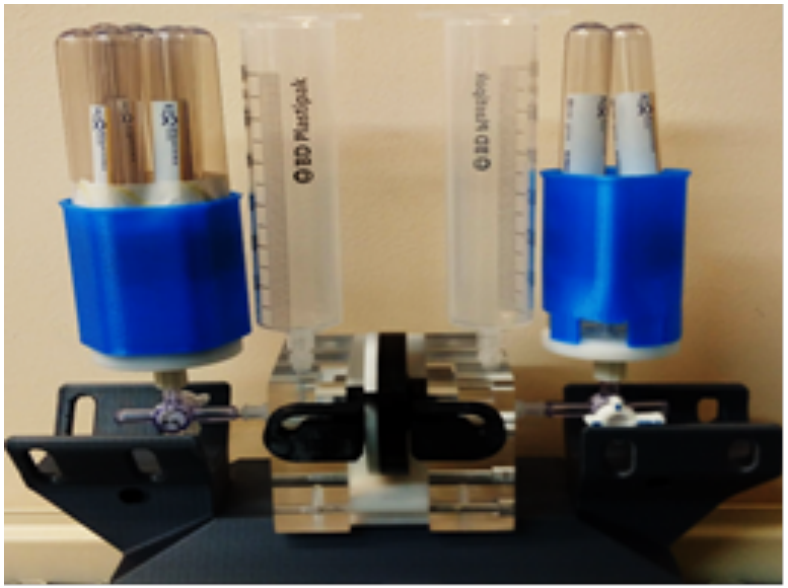
Development of a single use cell collection device

- Aims
  - Cell recovery equivalent to centrifugation
  - Filtration process did not compromise cellular integrity
  - Process completed with cells suspended in preservative

- Methodology
  - Urine from healthy volunteers spiked with bladder cancer cell line, RT112
  - Membranes of optimal pore size identified
  - Filtration pressures calculated for optimal flow of urine through membrane

Development of a single use cell collection device

- Device “powered” with readily available vacutainers
- Patented filter holder to allow filtration of urine sample
- Flow channels then allow cells to be suspended in preservative



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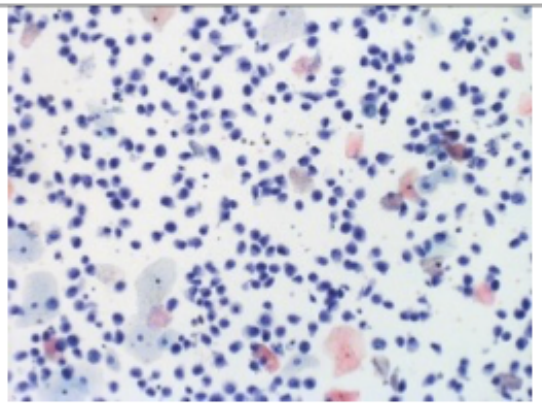
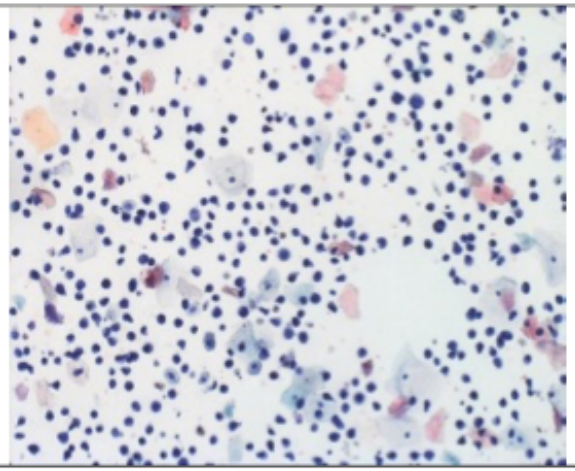
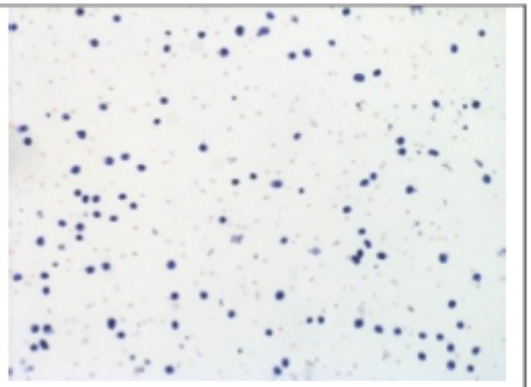
Development of a single use cell collection device

- Cellular capture of the device comparable to centrifugation
- Filtrate contains immune and blood cells
- Results in a “cleaner” sample for analysis

Centrifuge	Cell collection device	Filtrate
		

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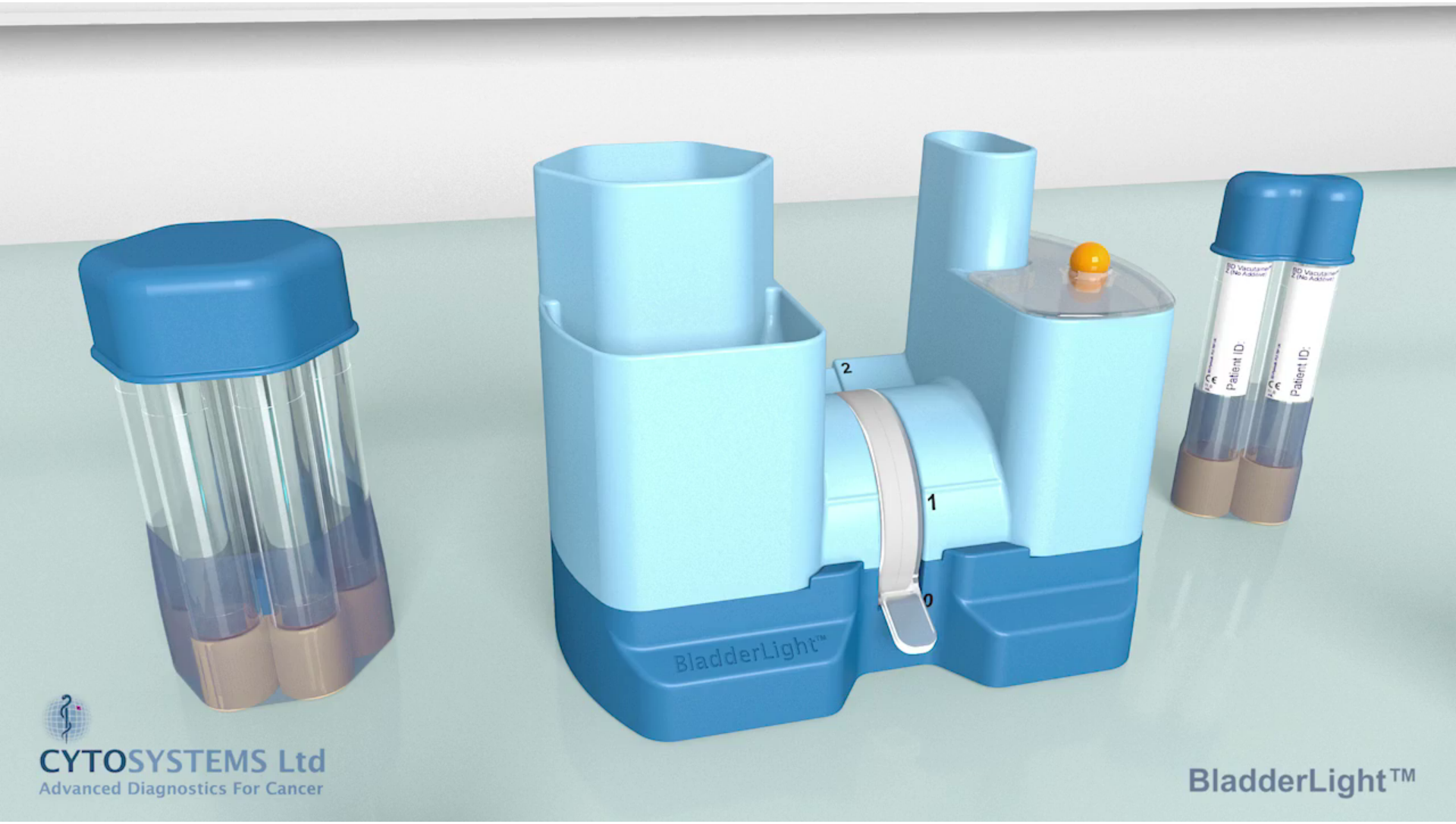
2
1
0
BladderLight™
Patient ID:
Patient ID:
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Clinical validation trial of the Bladderlight^{TM} system

- Assessment of Bladderlight SurvEILance (ABSEIL)
- Multi-centre clinical trial examining the accuracy of the Bladderlight test in the detection of bladder cancer
- 3 clinical questions:
  - Can the test detect bladder cancer recurrence in the surveillance group?
  - Can the test predict progression to overt disease?
  - Can the test detect the presence of bladder cancer at first presentation?

The potential positive impact of Bladderlight

- The potential to reduce the number of surveillance cystoscopies
- The potential to detect occult disease recurrence
- Single use device allows for point of care testing at GP surgery, at home

- Ultimately, this may lower the cost of managing bladder cancer − to the NHS as well as to the patient