Tier 3: The effectiveness of urinary biomarker genotypes (Cxbladder Detect) in the investigation of haematuria in Primary Care
National Health Committee (NHC)

The National Health Committee (NHC) is an independent statutory body charged with prioritising new and existing health technologies and making recommendations to the Minister of Health.

It was reformed in 2011 to establish evaluation systems that would provide the New Zealand people and the health sector with greater value for money invested in health.

The NHC Executive is the secretariat that supports the Committee. The NHC Executive’s primary objective is to provide the Committee with sufficient information for it to make decisions regarding prioritisation and reprioritisation of interventions and services. They do this through a range of evidence-based products chosen according to the nature of the decision required and timeframe within which decisions need to be made.

The New Zealand Government has asked that all new diagnostic and treatment (non-pharmaceutical) services, and significant expansions of existing services, are to be referred to the NHC.

In August 2011 the NHC was appointed with new Terms of Reference and a mandate to establish the capacity to assess new and existing health technologies. Its objectives (under Section 4.2 of its Terms of Reference – www.nhc.health.govt.nz) include contributing to improved value for money and fiscal sustainability in the health and disability sector by:

- providing timely advice and recommendations about relative cost-effectiveness based on the best available evidence;
- providing advice and recommendations which influence the behaviour of decision makers including clinicians and other health professionals;
- providing advice and recommendations which are reflected in resource allocation at national, regional and local levels; and
- contributing to tangible reductions in the use of ineffective interventions and improved targeting to those most likely to benefit.

In order to achieve its objectives under Section 4.2 and to achieve ‘Value for Money’, the NHC has adopted a framework of four assessment domains – Clinical Safety & Effectiveness; Economic; Societal & Ethical; and Feasibility of Adoption – in order that assessments cover the range of potential considerations and that the recommendations made are reasonable.

It is intended that the research questions asked will fall across these domains to ensure that when the Committee comes to apply its decision-making criteria, it has a balanced range of information available to it. When the NHC is setting those questions they will have the decision-making criteria in mind.

The 11 decision-making criteria will assist in the determination of the NHC work programme and in the appraisal and prioritisation of assessments.
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Executive Summary

The National Health Committee (NHC) has an identified role to “Pull” new technologies into the New Zealand health sector. This type of planned facilitation of new interventions is a new activity for the sector and the NHC has developed a relationship with Pacific Edge, a New Zealand based technology company to test how best to pull innovation into the system.

Pacific Edge has developed a number of new tests and diagnostic tools for the urothelial type of bladder cancer based on the use of urinary biomarkers – mRNA of genes implicated in tumour genesis. A number of these tools are currently used in secondary care as part of the diagnostic pathway.

The company has developed a new diagnostic which combines the urinary biomarkers used in the secondary care laboratory setting with patient clinical information (phenotypes) to develop a diagnostic tool for primary care.

The NHC has identified that this diagnostic could have significant impact on the diagnostic model of care for urothelial bladder cancer and that it also provides an opportunity for the Committee to test out the best ways to pull technology into use.

However, before progressing further the Committee wishes to understand the clinical safety and effectiveness of the genotypic components used in Pacific Edge’s Cxbladder Detect, as these genotypes are part of the newer Cxbladder Triage diagnostic tool that it wishes to jointly evaluate with Pacific Edge.

Cxbladder Triage has the potential to change the clinical model for the diagnosis of urothelial bladder cancer by enhancing diagnostic opportunities in the primary care setting. The use of Cxbladder Triage as a primary care diagnostic offers the potential to reduce demand for specialist urology services.

Urinary biomarker tests have varying effectiveness in detecting urothelial bladder cancer. Measures of their current performance show that all of them would miss a number of cases of urothelial cancer. A change in the diagnostic model introducing a urinary biomarker test as a sole gateway to referral, whether Cxbladder Detect or another, would potentially improve the detection rate of urothelial cancer in primary care as compared with existing methods of diagnosis. However as the sensitivity of the biomarkers is less than 100% some cases of bladder cancer would be missed.
Cxbladder Detect would not detect all cases of early stage, lower risk urothelial cancer while NMP22 and other alternative biomarkers would not detect all cases of early and late stage, lower and higher risk, urothelial cancer.

Urinary biomarkers offer opportunities to improve the diagnostic model for bladder cancer and Cxbladder Detect shows better performance over other urinary biomarkers.
1. Context

The role of biomarker tests in care of haematuria is outlined in the Tier 2 paper *Haematuria* (November 2014). Briefly, both cytology and cystoscopy have limitations in the detection of bladder cancer. Cytology requires specialist expertise, and has a relatively low sensitivity. Cystoscopy cannot detect upper urinary tract cancers, and is an invasive process. Biomarker tests are urinalysis tests and thereby non-invasive. They test for genetic (DNA and/or RNA) markers of urothelial carcinoma.

The National Health Committee (The Committee) considered the diagnostic model of care for haematuria (blood in the urine) at its September 2014 meeting. It had been previously agreed that the Executive perform a Tier 3 assessment of the potential role of urinary biomarkers in the diagnosis of urothelial cancer in the primary care setting and the impact on secondary care. At their September 2014 meeting, after consideration of the Tier 2 *Haematuria* report, the Committee decided that a Tier 3 assessment was needed of one particular composite biomarker test, *Cxbladder Triage*. In order to understand the role and utility of *Cxbladder Triage* it is necessary to firstly understand how the genotypic urinary biomarkers which are included in *Cxbladder Triage* perform in a routinely applied secondary care laboratory test. This test is called *Cxbladder Detect* and is assessed in this report with respect to safety and effectiveness.

*Cxbladder Detect* is a multi-gene test developed by Pacific Edge in New Zealand. It uses quantitative polymerase chain reaction (PCR) amplification to measure mRNA markers associated with urothelial cancer.\(^{(1)}\)

The technical information in this report will assist the Committee to understand the role and utility of *Cxbladder Triage* in primary care and help the Committee to understand some of the issues in pulling new technology into use.

2. Bladder Cancer

The epithelial lining of the urinary tract is consistent from the renal pelvis to the bladder. Most cancers that form in the bladder, the renal pelvises, the ureters, and the proximal urethra are urothelial transitional cell carcinoma (TCC) that derive from transitional epithelium.\(^{(2)}\) Urothelial bladder cancer’s cardinal symptom is painless, visible haematuria. A smaller group of patients present with irritative infection-like symptoms in the absence of visible haematuria;\(^{(3)}\) while some may present with microscopic haematuria alone.\(^{(4)}\)
2.1 Histological types
International data shows that approximately 90–95% of bladder cancer is caused by transitional cell carcinoma; the balance being mostly squamous cell carcinoma and adenocarcinomas.\(^5,\,6\) The other histological types of bladder cancer are clinically similar to TCC.\(^2\) TCC develops from the urothelium and can also occur in the ureters and renal pelvis.\(^6\)

2.2 Disease Stage
The stage of disease at diagnosis helps predict outcomes and direct clinical management; and progressive stages are associated with worsening 5-year survival rates.\(^7\)

Staging of bladder cancer uses the Tumour, Node and Metastasis (TNM) system, where ‘T’ refers to features of the primary tumour; ‘N’ to regional lymph nodes and ‘M’ to the presence of distant metastasis. Stages of bladder cancer are divided into non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC). NMIBC tumours are confined to the bladder urothelium (Ta and Tis) and to the lamina propria (T1) (see Figure 1) and account for the majority (75%) of newly diagnosed urothelial tumours.\(^6\)

2.3 Disease grade
NMIBC tumours of the urothelium are classified by their papillary structure and the variation in the degree of cellular proliferation and atypical cellular features. Carcinoma-in-situ (CIS or TIS) is a flat lesion with high grade malignant cytological features, and is usually multifocal and diffuse.\(^6\) The World Health Organization (WHO) recommends bladder cancer is graded as well-differentiated or low grade, and poorly-differentiated or high grade.\(^1\) Tumour grades are related to prognosis.\(^8\)

2.4 Risk factors
There are demographic and clinical risk factors for bladder cancer, which, together with clinical presentation are used in the diagnostic process. This process assesses a patient’s risk of having bladder cancer, and considers alternative diagnoses. As reflected in section 4.1 below, bladder

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\(^1\) American Joint Committee on Cancer (AJCC) Staging System
cancer is significantly more frequent in men than women. Other risk factors for developing bladder cancer include:\(^6\)

- age – there is a strong rise in incidence with increasing age;
- smoking – increases the risk of bladder cancer by up to four times;
- exposure to carcinogens – there is strong evidence for this link;
- occupational exposure – higher rates of bladder cancer are reported in textile dye and rubber tyre industries; among painters; leather workers; shoemakers; and aluminium, iron, and steelworkers;
- chronic cystitis is a risk factor for squamous cell carcinoma.

**Gender**

The impact of the gender of a patient on the risk of bladder cancer is well recognised. Primary care based studies of macroscopic haematuria show that urinary tract cancers are two to three times as common in men as women.\(^9\)\(^10\) In asymptomatic patients with microscopic haematuria the rate of urological cancer is higher in men.\(^11\) In a UK haematuria clinic urothelial cancer was one and a half times more common in men than women (15% v 10%).\(^12\)

**Macroscopic haematuria**

Secondary care studies of patients in haematuria clinics have found that urothelial cancer is around five times more common in people with macroscopic haematuria than in those with microscopic haematuria.\(^12\)-\(^14\) A systematic review of studies published between 1983 and 1995 of diagnostic features of urological cancers in which most cancers detected were urothelial cancer, found, that 19% of those with macroscopic haematuria were diagnosed with a urological cancer.\(^15\)

**Increasing age**

The frequency of urinary tract cancers increases with age and were shown to be 12 times more common in patients aged 75 or more compared to those aged less than 55 years.\(^9\) In those aged 40 or more years cancers were three and a half times more common than in those younger than 40 years.\(^15\) A primary care study of patients presenting with macroscopic haematuria found that 22.1% of men aged over 60 years had cancer compared with 14.2% of men of all age groups. In the men aged over 60 years, the presence of weight loss or fatigue increased the risk of malignancy 30% or more.\(^10\)
Smoking

In a secondary care haematuria clinic group of patients with macroscopic haematuria, urothelial cancer was 50% more common in current or ex-smokers compared to non-smokers. Smoking was also associated with higher grade and with more advanced disease at the time of diagnosis.\(^{(16)}\)

3. Current Diagnostic Practice

Haematuria may either be visible to the naked-eye (macroscopic) or invisible (microscopic) and detected with simple point-of-care urine test strips or by laboratory microscopy. There are several possible diagnostic explanations for haematuria, including urinary infection, renal calculi (stones), and glomerular renal disease; and consideration of other clinical features can aid diagnosis.

The cardinal symptom of urothelial bladder cancer is painless, visible haematuria. A smaller proportion of patients present with irritative infection-like symptoms in the absence of visible haematuria.\(^{(3)}\) Some patients may present with micro-haematuria alone; and patients with upper urinary tract urothelial carcinomas may present with pain due to obstruction by the tumour.\(^{(4)}\)

Clinical features do not differentiate between histological types of bladder cancer; clinical features are common to urothelial and non-urothelial cancers.\(^{(17)}\) A small study of patients with non-urothelial bladder cancer showed that patients presented with microscopic or macroscopic haematuria or other bladder symptoms.\(^{(18)}\) For different histological types mean age of presentation is in the 70s for urothelial cancer, squamous cell and neuroendocrine tumours and in the 60s for adenocarcinoma.\(^{(19)}\) The study of non-urothelial bladder cancers has been limited by their relative rarity.\(^{(19)}\)

4. Current Guidance

Following initial clinical assessment, further investigation may be through urinalysis, urine cytology, urinary tract imaging and flexible cystoscopy.\(^{(3)}\) A New Zealand clinical practice guideline recommends the assessment pathway shown in Figure 2.\(^{(20)}\)
Initial investigation of visible haematuria in any patient assessed as being at a higher risk of cancer (that is, macroscopic haematuria, over 40 years of age or other factors such as positive smoking history) when obvious causes have been excluded, includes imaging and cystoscopy. Computed tomography (CT) urogram is regarded as the current gold standard for imaging. However in primary care access to CT urogram is variable. Ultrasound and intravenous urogram have diagnostic roles in lower-risk patients. CT urogram combined with cystoscopy allows for both the direct examination of the bladder and assessment of the whole urinary tract.
causes of persistent and unexplained microscopic haematuria in high risk groups is also recommended.\(^{(20)}\)

A United Kingdom (UK) source of clinical guidance suggests referral for imaging and cystoscopy in the presence of macroscopic haematuria and in those over 40 years of age with microscopic haematuria.\(^{(21)}\) American Urological Association guidance for patients with asymptomatic haematuria recommends radiological imaging of the upper urinary tract followed by cystoscopic examination of the urinary bladder.\(^{(22)}\)

The role of voided urinary cytology is questioned for high risk patients who proceed to cystoscopy and radiology as the addition of cytology to this diagnostic pathway is unlikely to significantly increase the rate of cancer detection.\(^{(20)}\) Voided urinary cytology may be useful for patients at lower risk of urothelial cancer with asymptomatic haematuria.\(^{(22)}\)

Northern Region and Canterbury clinical protocols recommend the use of cytology and renal ultrasound for most patient groups with haematuria.\(^2\)

### 5. Current options in diagnostic investigation of haematuria in primary care

Initial clinical history and physical assessment, urine examination and blood tests of renal function can diagnose the non-malignant explanations for haematuria such as infection, calculi and intrinsic renal disease. Radiological imaging may be used to inform these clinical conditions further and the absence of a clear alternative diagnosis for the presentation of macroscopic haematuria or the persistence of microscopic haematuria. Persistent microscopic haematuria is defined as the presence of two out of three positive dipstick tests, seven days apart.\(^{(20)}\)

The next clinical step may be to refer to urological specialist assessment directly. This may be if the patient is considered at high risk of malignancy in view of age, smoking presentation and/or occupational history or through guidance recommendation. As noted above urinary cytology and urinary tract imaging are investigative options.

Diagnosis cannot rely solely upon urinary cytology, because although it is highly specific, its sensitivity is insufficient. It is less sensitive in detecting low grade disease.\(^{(23)}\)

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\(^2\) Personal communication
found a median sensitivity of 44% and specificity of 99% for cytology using voided urine samples. Analysis was of 3,331 participants in seven studies who were suspected of having bladder cancer.\(^{24}\)

That is, it produces a high proportion of false negative test results, so it will miss the majority of cases of malignancy. Cytology may be less effective in patients with microscopic haematuria. In a study of patients with asymptomatic microscopic haematuria with as bladder cancer prevalence of 4% no cases had positive cytology though half had atypical cytology. If atypical was taken as positive then sensitivity was 50% and specificity of 90%.\(^{25}\) Sensitivity has also been reported at 25%.\(^{14}\)

Ultrasound is a radiation-free imaging technique, usually readily available in the community and secondary care, and is considered appropriate first-line investigation for lower-risk patients. If these patients’ symptoms persist after negative initial investigations, they may need an intravenous urogram (IVU) or CT urogram.\(^{12, 26}\) IVU allows anatomical assessment of the whole urinary tract and can detect tumours, particularly renal tumours, from the presence of filling defects of the renal anatomy outline. CT urography has better detection rates for upper-tract urothelial cancers than ultrasound or IVU.\(^{3}\) However, CT urogram is not necessarily used as a first investigation because it is a limited resource, and because of the desire to limit patients’ exposure to radiation.

### 6. Diagnostic outcomes for haematuria

**6.1 Primary care**

There are various underlying causes for haematuria in patients presenting to primary care with haematuria alone or with other symptoms. The frequencies of diagnoses that explain the symptom(s) vary by age and sex of the patients. More patients with macroscopic haematuria will have bladder cancer diagnosed than those with microscopic haematuria. However, the rates vary across studies.

*Macroscopic haematuria*

Adults presenting with visible haematuria were identified in a retrospective analysis of a UK primary care clinical database of routinely completed clinical records. The records were analysed to determine the assumed consequent diagnosis. Six months after initial presentation, urinary tract malignancy was found to be an uncommon explanation for visible haematuria: 5.5% in men and 2.5% in women (4.1% combined).\(^{9}\)
A Belgian study of patients presenting with macroscopic haematuria and with at least 18 months follow-up found 10.3% of patients were diagnosed with urological cancer, 8.3% with bladder cancer and 2.0% other.\(^\text{10}\)

A United States (US) primary care study of 449 patients presenting with macroscopic or microscopic haematuria, of which 15% had macroscopic haematuria, showed that at 29 months of follow-up, bladder cancer was only diagnosed in the group with macroscopic haematuria at a rate of 6.7%.\(^\text{27}\)

**Microscopic haematuria**

A US study of patients with asymptomatic microscopic haematuria found bladder cancer was diagnosed in 0.2% of patients during 3 years of follow-up.\(^\text{11}\) Another US study of patients aged 18 years and over with microscopic haematuria had an incidence of bladder cancer of 0.68% during 3 years of follow up. The risk of urological cancer was greater in men than women and in those aged 40 years and over.\(^\text{28}\)

**6.2 Secondary care**

Malignant outcomes for patients with haematuria from studies performed in secondary care setting as shown in Table 1. The proportions of patients presenting with microscopic and macroscopic haematuria vary between studies.

<table>
<thead>
<tr>
<th>Population</th>
<th>Study period</th>
<th>N</th>
<th>Patients with macro-haematuria (%)</th>
<th>Bladder urothelial cancer (%)</th>
<th>Upper tract urothelial cancer (%)</th>
<th>Renal cell carcinoma (%)</th>
<th>No formal diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Australia(^\text{29})</td>
<td>2008–11</td>
<td>500</td>
<td>60</td>
<td>10.2</td>
<td>1.0</td>
<td>1.0</td>
<td>69</td>
</tr>
<tr>
<td>UK(^\text{12})</td>
<td>1998–2003</td>
<td>4023</td>
<td>52</td>
<td>10.3</td>
<td>0.3</td>
<td>1.5</td>
<td>80</td>
</tr>
<tr>
<td>UK(^\text{30})</td>
<td>1999</td>
<td>363</td>
<td>48</td>
<td>7.7</td>
<td>–</td>
<td>1.4</td>
<td>72</td>
</tr>
<tr>
<td>UK(^\text{11})</td>
<td>1994–97</td>
<td>1,930</td>
<td>49</td>
<td>11.9</td>
<td>0.1</td>
<td>0.6</td>
<td>61</td>
</tr>
<tr>
<td>UK(^\text{14})</td>
<td>1993–98</td>
<td>1046</td>
<td>37</td>
<td>8.4</td>
<td>1.0</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>UK(^\text{16})</td>
<td>1999-2007</td>
<td>1804</td>
<td>100</td>
<td>18</td>
<td>0.4</td>
<td>2.2</td>
<td>49</td>
</tr>
</tbody>
</table>

**Source:** 2014 NHC Executive literature search

The presented studies cover different time periods that may have an impact on the described results as one study noted the change from investigating patients predominantly with macroscopic to those with predominantly microscopic haematuria over time.\(^\text{12}\) One study included only patients with...
visible haematuria\(^{(16)}\) and detected a greater frequency of malignancies (20.6%) than the other studies.

Bladder cancer was found in about 8%–12% of patients. Urothelial cancer was found in around 8%–11% of patients.\(^{(12,14,29,32)}\) The frequency of detection of malignancy is higher with macroscopic presentation than microscopic; 18%\(^{(16)}\) 18% versus 5%,\(^{(12)}\) 25% versus 4%,\(^{(14)}\) 20% versus 5%,\(^{(31)}\) and 24% versus 2%.\(^{(32)}\) In one study of the 90 cases of bladder cancer the urothelial histological type accounted for 88 cases (98%).\(^{(14)}\) No specific disease is diagnosed in between 70% and 80% of patients presenting with haematuria.

Diagnostic outcomes for patients presenting with microscopic haematuria, when specifically identifiable, in primary and secondary care settings are shown in Table 2. Renal cancer outcomes and urothelial cancer outcomes are shown when available.

<table>
<thead>
<tr>
<th>Table 2: Diagnostic outcomes of patients with microscopic haematuria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Population</strong></td>
</tr>
<tr>
<td>----------------</td>
</tr>
<tr>
<td>UK(^{(12)})</td>
</tr>
<tr>
<td>UK(^{(30)})</td>
</tr>
<tr>
<td>UK(^{(31)})</td>
</tr>
<tr>
<td>UK(^{(14)})</td>
</tr>
<tr>
<td>US(^{(28)})</td>
</tr>
</tbody>
</table>

Source: 2014 NHC Executive literature search

### 7. Need for an NHC assessment

Current options for diagnosis of urothelial cancer include cytology and cystoscopy. Cytology does not perform well in detecting malignancy. It has a sensitivity of 40% to 60% with significant inter-observer variability, and needs interpretation by specialists.\(^{(33)}\) Cystoscopy is invasive\(^{(23)}\) and cannot alone detect upper tract cancers for which imaging modalities are needed.

Cystoscopy is the standard tool for the diagnosis of bladder cancer and the ‘gold standard’ and often the comparator in the biomarker studies. However, its diagnostic performance is not perfect.
White-light cystoscopy (WLC) is the standard method and it can miss small papillary tumours and carcinoma-in-situ (CIS). Photodynamic diagnosis (PDD) improves detection as does narrow-band imaging (NBI) using a shorter wavelength source.\(^7,34\) A systematic review that included 27 studies and 2,949 patients compared the performance of WLC and PDD. At patient level analysis WLC had a sensitivity of 71% and specificity of 72% whereas PDD had sensitivity of 92% and specificity of 57%.\(^{35}\) A systematic review of good quality studies produced pooled estimates for NBI cystoscopy and WLC with sensitivities of 94% and 85% for NBI and WLC respectively.\(^{36}\)

Alternative diagnostic options may offer a reduced need for invasive investigation.

### 7.1 Urinary Biomarkers testing

Testing for urinary biomarkers offers an alternative tool in diagnosis of haematuria. Tests for urinary biomarkers are non-invasive and could potentially overcome the limitations of current diagnostic options. If effective in the primary care setting, they could identify patients without urothelial cancer, and avoid specialist referral and further investigation.

The molecular basis for the development of urothelial cancer appears to have two pathways. The more common involves chromosomal deletions and activation of fibroblast growth factor receptor 3 (FGFR3) gene and the more aggressive pathway involves chromosomal deletions and gene mutations. In the latter pathway the cells demonstrate retinoblastoma (RB) inactivation and defects in H-ras.\(^6\) The Genetic Home Reference of the National Institute of Health reports several genes including FGFR3, RB1, HRAS, TP53, KRAS and TSC1 have a role in the formation and growth of bladder tumours,\(^3,4\) although others show differential expression in bladder cancer tissue compared to normal tissue.\(^{37-5}\)

Urothelial cancer-related change produces genetic (DNA and mRNA) markers in urine,\(^6\) and if these markers are detected in urine samples, they offer a potential means of diagnosis for urothelial cancer. Different tests and methods applied to urinary samples for diagnosis include:\(^{23}\)

- **UroVysion**: uses FISH (fluorescence in situ hybridisation) to detect chromosomal anomalies on chromosomes 3, 7, 9 and 11. It detects the most common chromosomal abnormalities in urothelial cancer.\(^{38}\)
• **Immunocyt**: uses immunocytochemistry to visualize the plasmic mucins and carcinoembryonic tumour-associated antigens associated with urothelial carcinoma cells exfoliated in voided urine\(^{(39)}\)

• **Telomerase repeat amplification protocol (TRAP) assay**: determination of telomerase activity using polymerase chain reaction. Telomeres are sequences at the end of chromosomes that protect genetic stability during cell division. Bladder cell cancers express telomerase that allows ongoing cellular replication

• **BTA-TRAK**: The assay is a quantitative enzyme immunoassay. The antigen measured is human complement factor H-related protein (hCFHrp). In cell culture, hCFHrp has been shown to be produced by several human bladder cancer cell lines but by not healthy epithelial cells\(^{(40)}\)

• **Hyaluronic acid (HA) and hyaluronidase (HAase) assay**: HA levels are higher in tumour cells, including those in bladder tumours. HAase is elevated in bladder tumour tissue and is correlated with tumour grade

• **Nuclear Matrix Protein 22 (NMP22)**: a protein that is important for the regulation of mitosis. NMP22 is elevated in tumour cells and is released allowing detection. It is found at significantly higher levels in the urine of patients with TCC\(^{(38)}\)

• **BCLA-4**: a nuclear matrix protein that is overexpressed in bladder cancer. It is tested with an enzyme-linked immunosorbent assay (ELISA)

• **Cytokeratins**: filaments that enable cells to withstand mechanical stress. Certain subtypes are associated with bladder cancer

• **Survivin**: a protein involved in the regulation of cell death. Excess levels promote cell survival. It is detectable in urine of patients with bladder cancer

• **Epidermal Growth Factor Receptor (EGFR)**: overexpressed by bladder cancer tumours. This protein plays an important role in the growth, proliferation and differentiation of numerous cell types.\(^{7}\)

The US Food and Drug Administration (FDA) has approved a number of these tests including **BTA stat, BTA TRAK, NMP22, Immunocyt and UroVysion**. The performance of both **BTA** and **NMP22** is affected by the degree of haematuria. **Immunocyt** detects cellular biomarkers on cytology slides with fluorescent antibodies and a trained cytopathologist is needed to read the test result. It is prone to inter-observer variation but is less affected by the presence of blood and inflammatory conditions.

UroVysion is intended to be used in conjunction with, rather than instead of, other diagnostic procedures.\(^{(41)}\) The use of FISH is considered limited by its high costs and workload.\(^{(23)}\)

Mutations in individual genes may not be common across urothelial cancers and so the detection of mutations in more than one gene offers a higher detection rate and enhanced performance. Multiplex markers are at an early stage of development. The combination of multifaceted molecular tests and clinical criteria offers promise to give the desired clinical utility.\(^{(42)}\)

### 7.2 Cxbladder Detect urinary biomarkers and Cxbladder Triage

*Cxbladder Detect* is a multi-gene test developed by Pacific Edge in New Zealand. It uses PCR amplification to quantify mRNA markers of genes associated with urothelial cancer.\(^{(1)}\)

*Cxbladder Detect* does not analyse the genes of the bladder epithelium directly. The diagnostic test measures gene transcription and quantifies mRNA for five markers detectable in voided urine. Of the five markers, four are overexpressed in urothelial cancer and a fifth is over-expressed in non-malignant inflammatory conditions.\(^{8}\) From the urine sample mRNA is quantified for the genes IGFBP5, HOXA13, MDK, CDK1 and CXCR2.

The genes whose activity is quantified with mRNA in the *Cxbladder Detect* are not specific to the currently known pathogenic pathway for urothelial cancer but are genes that have roles in the cellular regulation and cancer development:

- IGFBP5 is a protein-coding gene and plays a role in cancer by regulating cell motility and cell survival\(^{(9,43)}\)
- HOXA13 encodes a DNA-binding transcription factor which may regulate gene expression, morphogenesis, and differentiation\(^{(10)}\)
- MDK gene encodes a protein that promotes cell growth and migration particularly during tumour development\(^{(11)}\)
- CDK1 encodes for a protein that plays a key role in the control of the eukaryotic cell cycle\(^{(12)}\)
- CXCR2 (chemokine receptor 2) is a protein-coding gene. Chemokines belong to a family of molecules involved in the directed migration of immune cells.\(^{(13)}\)

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9 The Human Gene Compendium: [http://www.genecards.org/cgi-bin/carddisp.pl?gene=IGFBP5](http://www.genecards.org/cgi-bin/carddisp.pl?gene=IGFBP5)


11 The Human Gene Compendium: [http://www.genecards.org/cgi-bin/carddisp.pl?gene=MDK&search=f965ea4a8be32190599bdc20f2a3424e50](http://www.genecards.org/cgi-bin/carddisp.pl?gene=MDK&search=f965ea4a8be32190599bdc20f2a3424e50)

12 The Human Gene Compendium: [http://www.genecards.org/cgi-bin/carddisp.pl?gene=CDK1](http://www.genecards.org/cgi-bin/carddisp.pl?gene=CDK1)

The underlying genes incorporated into Cxbladder Detect are not common to other currently identified urinary biomarkers.

Cxbladder Detect is already used in secondary care to assist in diagnosis. The urinary biomarkers in Cxbladder Detect have been used as part of a new diagnostic tool for haematuria in primary care, Cxbladder Triage. These biomarkers combined with phenotypic information (observable patient characteristics such as age, gender and smoking history) are used to provide a risk stratification tool to assist the primary care clinician to understand the appropriate diagnostic and referral pathway to follow.

Urinary biomarkers have the potential to change the model for the diagnosis of urothelial cancer in the primary care setting with associated impacts on secondary care. In order to understand the utility of Cxbladder Triage, it is necessary to understand the effectiveness and safety of the urinary biomarkers which are used in the tool and so the NHC decided that further analysis of Cxbladder Detect is warranted.

### 7.3 Target group for genetic urinary marker testing

Clinical assessment of bladder cancer risk is based on identification of alternative clinical explanations for haematuria, whether the blood is visible or invisible, the presence of other symptoms and presence of risk factors. Clinical uncertainty about the underlying condition together with risk factors for underlying malignancy drives the need for further investigation.

The target group for genetic biomarker testing are patients who present in primary care with haematuria, and who are considered at high risk of an underlying bladder cancer either through presenting with visible haematuria or because they have persistence of invisible haematuria and do not have an alternative explanation for their presentation despite investigation to exclude other identifiable causes.

**Assessment**

This assessment analyses the safety and effectiveness urinary biomarkers as part of the diagnostic pathway for urothelial cancer.
8. Clinical safety and effectiveness

8.1 Methods

Research Question

The formal research question for the assessment of safety and effectiveness is:

In people with haematuria in primary care who would otherwise be referred to secondary care for specialist assessment, is the use of the urinary biomarker components of Cxbladder Detect a safe and clinically effective tool for diagnosing urothelial cancer, compared with current standard investigations?

Literature review

A systematic search for evidence was performed, limited to English language as shown in Table 3.

<table>
<thead>
<tr>
<th>Table 3: Evidence Search</th>
</tr>
</thead>
</table>
| **Cochrane library**     | biomarkers (under urology)  
cystoscopy (under urology) |
| **Medline**              | systematic reviews: MESH terms [bladder cancer]  
[biological markers]  
systematic reviews: MESH terms [bladder cancer/diagnostics] [cystoscopy] |
| **Centre for Reviews and Dissemination** | MESH terms [bladder cancer] [biological markers] |
| **MEDLINE, Embase, Scopus, Cochrane Library** | MESH terms [Hematuria] and [Primary Health Care] or [Family Practice/ or General Practice] or [general practitioners/ or physicians, family/ or physicians, primary care]  
MESH terms [urinary bladder neoplasms] (non-transitional or non-urothelial or non-transitional or non-urothelial) and (transitional or urothelial) |

Studies retrieved following the search strategy were appraised and data were extracted. Further relevant references from the appraised articles were retrieved for assessment.
Measurement of diagnostic test performance

Performance of diagnostic tests is measured by their sensitivity (the ability to correctly detect the disease) and specificity (the ability to detect only the disease). Sensitivity is important because it describes the test’s ability to ‘find’ cases of the condition being tested for, while specificity is important because it describes the diagnostic test’s ability to correctly ‘rule out’ the condition.

Other measures of test performance are positive predictive value (PPV) and negative predictive value (NPV). These consider the performance for the perspective of the test result: PPV the proportion of patients who have a positive test with the condition of interest and NPV the proportion of negative tests without the condition of interest. Sensitivity and specificity are considered constant features while PPV and NPV are dependent on how common the condition is within the test population. While the sensitivity and specificity remain constant, as the condition of interest becomes more common in the test population the PPV increases and as the condition of interest becomes less common the NPV increases. In the situation when a diagnostic test has a high PPV a positive result ‘rules in’ a diagnosis and when the NPV is high a negative result ‘rules out’ a diagnosis. In the context of Cxbladder Detect where the default position without testing is to have a cystoscopy the NPV is the more relevant measure and needs to be high, thereby identifying true negative tests results with confidence, to stratify patients and thereby avoid the next step of invasive investigation. Cxbladder Detect combines the input of multiple markers via a mathematical algorithm to produce a final dichotomous result - positive or negative. As such, its diagnostic performance can be considered in terms of sensitivity/specificity and PPV/NPV in the test population.

8.2 Quality of evidence

The diagnostic tests are all performed on urinary samples but use a range of assay methods to detect the biomarker/s of interest. Operational features of the diagnostic tests are shown in Table 4 below.
Table 4: Features of urinary biomarker tests for diagnosis of urothelial cancer

<table>
<thead>
<tr>
<th>Test</th>
<th>Biological target</th>
<th>Sample</th>
<th>Assay used in studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder Tumour Fibronectin(^{(44)})</td>
<td>glycoprotein</td>
<td>Urine</td>
<td>Immunoassay</td>
</tr>
<tr>
<td>BTA stat(^{(23)})</td>
<td>protein</td>
<td>Urine</td>
<td>Immunoassay</td>
</tr>
<tr>
<td>BTA TRAK(^{(23)})</td>
<td>protein</td>
<td>Urine</td>
<td>Immunoassay</td>
</tr>
<tr>
<td>FISH(^{(23)})</td>
<td>chromosomal</td>
<td>Urine</td>
<td>Fluorescence</td>
</tr>
<tr>
<td>ImmunoCyt(^{(23)})</td>
<td>exfoliated cell antigens</td>
<td>Urine</td>
<td>Immunocytology</td>
</tr>
<tr>
<td>NMP22(^{(45)})</td>
<td>nuclear matrix protein</td>
<td>Urine</td>
<td>Immunochromatographic assay</td>
</tr>
<tr>
<td>Survivin(^{(46)})</td>
<td>mRNA and protein</td>
<td>Urine</td>
<td>Bio Dot microfiltration(^{14}) Reverse Transcription Polymerase Chain Reaction, Molecular Beacon fluorescence(^{15})</td>
</tr>
<tr>
<td>Cxbladder Detect(^{(1)})</td>
<td>mRNA</td>
<td>Urine</td>
<td>Reverse Transcription Polymerase Chain Reaction</td>
</tr>
</tbody>
</table>

Source: 2014 NHC Executive literature search

The evidence for the various tests’ performance is of varying quality and somewhat inconsistent. The studies included in a systematic review\(^{(35)}\) investigated a different balance of population groups between initial diagnostic and surveillance. A systematic review and meta-analysis of studies published between 1997 and 2011 on Survivin showed the original studies had high heterogeneity, limited blinding and poor reporting of selection criteria, but the same reference standard of cystoscopy was used. The studies’ use of healthy controls improved demonstrated specificity. For the majority of the studies it was not possible to determine whether the test was being applied for the purposes of initial diagnosis, surveillance or a preliminary study of accuracy.\(^{(46)}\) A systematic review of five studies of Bladder Tumour Fibronectin published between 1982 and 2012 showed the studies had a total of 649 patients receiving the test with 291 controls. The source and presentation of the patients and the nature of the control intervention were not clear and the authors noted that it was not possible to gather enough evidence to confirm the test’s role in early diagnosis.\(^{(44)}\) Small retrospective studies have been performed on cytokerin 20 using non-cancer urological patients and healthy volunteers as controls.\(^{(47,48)}\)

\(^{15}\) http://www.molecular-beacons.org/MB_introduction.html
Reviews of the state of biomarkers technology in 2011 and 2013 concluded that the available tests show promise but do not currently work well enough to replace invasive diagnostic methods.\(^{38, 41}\)

### 8.3 Performance of urinary biomarker tests

One systematic review analysed the performance of urinary biomarker tests for detecting bladder cancer. It included randomised controlled trials (RCTs), non-randomised comparative studies and diagnostic cross-sectional studies published up to April 2008. The studies included patients who were at risk of bladder cancer and who had a previous history of bladder cancer. The review included:\(^{35}\)

- 14 studies of *FISH*, with a total \(n\) of 3,321;
- 10 studies of *ImmunoCyt* with a total \(n\) of 4,199; and
- 41 studies of *NMP22* with a total \(n\) of 22,260.

Pooled estimates for sensitivity and specificity of biomarker tests are shown in Table 5. Data for other tests are from an alternative, non-systematic review.\(^{6}\) The table also shows the size of the study population that generated the summary measure and the proportion of participants that were part of trial and whether the test was used for the initial diagnosis of at risk patients.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Summary Sensitivity (%)</th>
<th>Summary Specificity (%)</th>
<th>Number of study participants</th>
<th>Used for initial diagnosis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bladder Tumour Fibronectin(^{44})</td>
<td>81</td>
<td>80</td>
<td>940</td>
<td>0</td>
</tr>
<tr>
<td>BTA stat(^{6})</td>
<td>68</td>
<td>74</td>
<td>Not Known</td>
<td>Not Known</td>
</tr>
<tr>
<td>BTA TRAK(^{6})</td>
<td>61</td>
<td>71</td>
<td>Not Known</td>
<td>Not Known</td>
</tr>
<tr>
<td>FISH(^{35})</td>
<td>76</td>
<td>85</td>
<td>3,321</td>
<td>45</td>
</tr>
<tr>
<td>ImmunoCyt(^{35})</td>
<td>84</td>
<td>75</td>
<td>4,199</td>
<td>27</td>
</tr>
<tr>
<td>NMP22(^{35})</td>
<td>68</td>
<td>79</td>
<td>13,885</td>
<td>41</td>
</tr>
<tr>
<td>Survivin(^{46})</td>
<td>75</td>
<td>94</td>
<td>2,047</td>
<td>1</td>
</tr>
<tr>
<td>Cxbladder-Detect(^{1})</td>
<td>82</td>
<td>85</td>
<td>485</td>
<td>100</td>
</tr>
</tbody>
</table>

Source: 2014 NHC Executive literature search

Although the pooled sensitivity of *ImmunoCyt* is better than that of *NMP22*, the included studies investigated a different balance of population groups. For *ImmunoCyt*, 73% of the patients (10% of studies) had previously been diagnosed with bladder cancer and only 27% were included because of suspicion of bladder cancer. For the *NMP22* studies, 41% of patients were included because of
suspicion of bladder cancer and for the FISH studies, 45% of patients because of suspicion of bladder cancer.\textsuperscript{(24)} For NMP22 the sensitivity for detecting higher risk patients (stage T1, grade 3 and CIS) was 83%, but only 50% for the lower risk patients (stage Ta, grades 1 and 2). For Immunocytcyt the sensitivity for higher risk patients was 90% and for lower risk patients was 81%\textsuperscript{(24)}.

\textit{Cxbladder Detect}

\textit{Cxbladder Detect} uses an algorithm to combine the results of five mRNA markers detected using polymerase chain reaction (PCR). Its performance has been assessed with reference to its detection of urothelial cancer. Its performance in the detection of other types of bladder cancer is not known. However, as it uses indicators of abnormal cellular turnover and control these other cancers may also be detectable.

The test is compliant with the New Zealand Medical Device Database and was registered in 2011\textsuperscript{16}. In 2013, the New Zealand laboratory of Pacific Edge Diagnostics (manufacturers of the test) obtained registration to test the performance of \textit{Cxbladder Detect} under the Clinical Laboratory Improvement Amendments (CLIA) regulatory process in the USA.

In the study of \textit{Cxbladder Detect} participants were patients who had presented to secondary care with macroscopic haematuria. Comparison diagnostic tests were cystoscopy (as the ‘gold standard’), NMP22 assays and cytology. The prevalence in the participants of urothelial cancer was 13.6% and 1.4% of other cancer types. The study reported \textit{Cxbladder Detect} had an overall sensitivity of 82% and specificity of 85%; with 100% sensitivity for stages T1–T3 disease, the more advanced stages and for CIS; and 68% sensitivity for stage Ta (56% of cases). 62 positive \textit{Cxbladder Detect} test results were for cases that were negative for urothelial cancer on initial clinical assessment; of these, three were subsequently shown to have urothelial cancer, 2 involving the upper tract. \textit{Cxbladder Detect} had better sensitivity than NMP22 (sensitivity = 69%).\textsuperscript{(1)}

The performance of \textit{Cxbladder Detect} has been compared to cytology, NMP22 and FISH across five datasets of either primary detection after presentation with macroscopic haematuria or secondary monitoring. The new dataset comprised 939 patients, missing data was inputted by alternative methods and cystoscopy was the required method to confirm the diagnosis of urothelial cancer. In the comparative analysis \textit{Cxbladder Detect} showed significant advantage over other tests in the detection of urothelial cancer. Sensitivity for \textit{Cxbladder Detect} was 80% whereas sensitivity ranged from 40% to 45% for the alternatives.\textsuperscript{(49)}

\textsuperscript{16} http://www.pacificedgedx.com/about-us/quality-assurance/
Combination testing

Combination testing has also been assessed. In a small study of patients with urothelial and non-urothelial bladder cancer, compared with others with non-malignant urological disease and normal subjects, the benefit of adding biomarkers tests to cytology was compared with use of cytology alone. The addition of Survivin to cytology increased sensitivity from 46% to 92%, and the further addition of CK-20 increased it to 96%. Using three different biomarkers tests by adding Mucin 7 (MUC7) produced a sensitivity of 100%. The three biomarkers tests detected squamous cell carcinoma as well as urothelial cancer. However these results should be treated with caution, as a systematic review of Survivin estimated its sensitivity at 75% (compared with the 90% in this study), and had concerns about the quality of the reviewed studies. Nonetheless, it suggests potential benefit from the use of more than one biomarker test particularly if this approach gives detection across different types of bladder cancer.

Another study analysed the diagnostic performance of combined tests in 808 patients who had haematuria or irritative symptoms and in whom urothelial cancer was suspected. Standard investigation was with cystoscopy and upper tract imaging. The combined tests were cytology with FISH, Immunocyt and NMP22. Performance was not measured by sensitivity alone, but analysed using Receiver-Operator-Curves (ROC), with description of the sensitivity, specificity and diagnostic algorithms at the best ROC level. The 68% sensitivity of cytology alone in this study was higher than usually found. The study found that adding a second test improved performance: adding FISH increased sensitivity to 78%, while the combination of FISH, Immunocyt and NMP22 increased sensitivity to 84%. Although this was the same as NMP22 alone in this study, the combination was much more specific at 74% compared with 41% for NMP22 alone.

Cxbladder Detect is a multi-item test. The performance of Cxbladder Detect has not been examined in conjunction with other diagnostic biomarker tests.

Summary of performance

The available sensitivities and specificities shown in Table 5 indicate that Cxbladder Detect has better sensitivity than some biomarkers tests, and better specificity than biomarkers tests that have similar sensitivity. Studies of urinary biomarkers have included patients at risk of urothelial cancer and those undergoing surveillance of recurrence of diagnosed and treated disease. The proportion of diagnostic patients in studies is higher for NMP22 at 41% and Cxbladder Detect at 100%.

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17 Personal communication. 2014
However, as noted above, not all cases of urothelial cancer are detected by biomarkers. The non-detected patients are at a lower stage of disease severity when *Cxbladder Detect* is used compared to NMP22 and other alternatives.

**Implications of non-detected cancer**

There is no known data on the risk of disease progression for people with missed diagnoses, but we can extrapolate from data on patients who have been diagnosed, staged and treated. Studies of treated patients show that several factors affect disease recurrence and progression to invasive bladder disease, including grade and stage of disease and number of tumours.\(^{(52)}\) Risk of progression is:

- 0.2% after a year for lowest risk patients, including those with low grade disease and stage Ta;
- about 1% at 12 months post-treatment for those with carcinoma in-situ;
- 9% risk of progression to invasive disease at five years for patients with Ta or T1 disease without CIS treated solely with TURBT;
- between 7 and 40% at 5 years for patients with Ta or T1 disease with CIS.

These data suggest that, in low risk disease, a delay of a few months would probably not be associated with disease progression to a state where treatment options are fundamentally changed.

However the impact on clinical outcomes of a delay, of for example a number of months, in diagnosis of bladder cancer should also be considered in the context of current practice. Time to diagnosis includes the time from presentation in primary care, through initial investigation, referral to secondary care and waiting time until the specialist assessment. If the introduction of an alternative diagnostic pathway reduced the overall time to diagnosis, a delay of some months for a subset of patients may not make a significant difference from the status quo.

The non-detection and consequent delay in diagnosis of patients with higher stage disease is associated with greater risk of progression to a disease stage that affects treatment options and outcomes.
9. Translating Cxbladder Detect into Cxbladder Triage

Pacific Edge has published a study on a new diagnostic clinical tool, Cxbladder Triage, that is expected to be used in the primary care setting. This test combines data from laboratory results, the genotypic data, and phenotypic data to calculate risk for the tested patients. This approach it is reported improves the test performance, including sensitivity in detecting urothelial cancer.\(^{(54)}\)

The genotypic and phenotypic data that Cxbladder Triage incorporates have been obtained from patients presenting with macroscopic haematuria in a secondary care setting. Validation is needed in a patient group representative of the primary care population to inform its applicability in a less selected clinical population.

10. Conclusions

Urinary biomarker tests have varying effectiveness in detecting urothelial cancer and their current known performance show that all would miss cases. A change in the diagnostic model to introduce a urinary biomarker test as a sole gateway to referral, would lead to missing or delayed cases of bladder cancer that would be detected under the current process of default referral and cystoscopy. NMP22 would miss more urothelial cancer cases than Cxbladder Detect. NMP22 would miss both lower and higher risk TCC cases while in comparison the cases not detected by Cxbladder Detect would be those with lower risk disease. Biomarker use in the primary care setting presents an opportunity to significantly change the diagnostic model for bladder cancer. However the performance of the available tests needs to be demonstrated in the appropriate setting to give support to the potential.
References


35. Mowatt G, Zhu S, Kilonzo M, Boachie C, Fraser C, Griffiths TR, et al. Systematic Review of the Clinical Effectiveness and Cost-Effectiveness of Photodynamic Diagnosis and Urine Biomarkers (Fish,
National Health Committee – Tier 3: The effectiveness of urinary biomarker genotypes (Cxbladder Detect) in the investigation of haematuria in Primary Care


52. Sylvester RJ, van der Meijden AP, Oosterlinck W, Witjes JA, Bouffioux C, Denis L, et al. Predicting Recurrence and Progression in Individual Patients with Stage Ta T1 Bladder Cancer Using


National Health Committee (NHC) and Executive

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