Dear Dr. Mann,

We are writing in response to your open request for comment on DL39365. This letter also addresses the draft LCD from First Coast Service Options. We have numerous concerns regarding the LCD, but most critically, the evidentiary review associated with the non-coverage determination of Cxbladder products.

EXECUTIVE SUMMARY:

- This letter contains Pacific Edge's response to the LCD for our Cxbladder Triage (0363U) and Detect (0012M) tests which are indicated for the hematuria evaluation in patients with no prior diagnosis of urothelial carcinoma (UC) as well as the Cxbladder Monitor test (0013M) which is indicated for surveillance of patients diagnosed with non-muscle invasive bladder cancer (NMIBC).
- We maintain that our published clinical data supports the inclusion of Cxbladder Triage and Detect for specific patient populations in the clinical pathway for hematuria evaluation, and the published clinical data supports the inclusion of Cxbladder Monitor into the clinical pathway for surveillance of patients diagnosed with non-muscle invasive bladder cancer(1-4).
- The letter also references three Cxbladder tests (Cxbladder Resolve, Enhanced Detect, and Enhanced Triage) referenced in the LCD that are in development and not commercially available and therefore are not appropriate for an evidentiary review or inclusion/exclusion from coverage(5).
- We share our medical rebuttal to many of the points made in the evidentiary review on the LCD that we believe do not reflect the clinical value of the tests and the substantial clinical evidence developed to validate them.
- Pacific Edge respectfully makes the following requests for changes to the LCD.

REQUESTS

1. ***We request Cxbladder Triage, Detect, and Monitor be included as covered tests in the final LCD language for the specific patient populations outlined below. The published clinical evidence and the demonstrated real world clinical value of these tests with high negative predictive value affirms the need for continued access of these tests to the Medicare population (see appendix for specific evidence).***
2. ***If Novitas does not support the request above:***
    a. ***We request that all tests in the hematuria evaluation pathway be completely removed from this LCD as they do not fit the inclusion criteria which requires an***

established diagnosis or significant suspicion of cancer. This removal would include Cxbladder Triage and Detect.

b. ***We request that once removed from LCD DL39365, Cxbladder Triage and Detect continue to be covered per the guide and documentation requirements of LCA 58917 as currently covered when the tests are documented as medically necessary by the treating physician.***
c. ***We request that Novitas convene a Contractor Advisory Committee session to determine if urinary biomarkers should be included in an existing or new LCD.***

3. ***We request that all mentions of Enhanced Cxbladder Detect, Resolve or Enhanced Cxbladder Triage be removed from the LCD as these tests are not available for clinical use. The data supporting the analytic validity, clinical validity, and clinical utility of these tests is still under development.***

SUPPORT FOR REQUESTS:

Support for Request #1

We request Cxbladder Triage, Detect, and Monitor be included as covered tests in the final LCD language for the specific patient populations outlined below. The published clinical evidence and the demonstrated real world clinical value of these tests with high negative predictive value affirms the continued access of these tests to the Medicare population.

Rationale:

<u>Cxbladder Detect</u>

Clinical Scenario and Patient Population

Cxbladder Detect is intended for use with patients presenting with any microhematuria to risk stratify those patients into low, intermediate, and high risk of bladder cancer. This stratification can reduce the burden of investigations for the low and intermediate risk patients after shared decision making with the patient and prioritize those with high risk for full investigation. The test can also be used to adjudicate diagnostic dilemmas when cytology is equivocal, or cystoscopy is un-informative in both microscopic and gross hematuria patients.

There are approximately 7 million patients in the US that present annually with hematuria, of which 75% present with microhematuria (defined as ≥3 RBC/HPF and with no visible blood in urine).

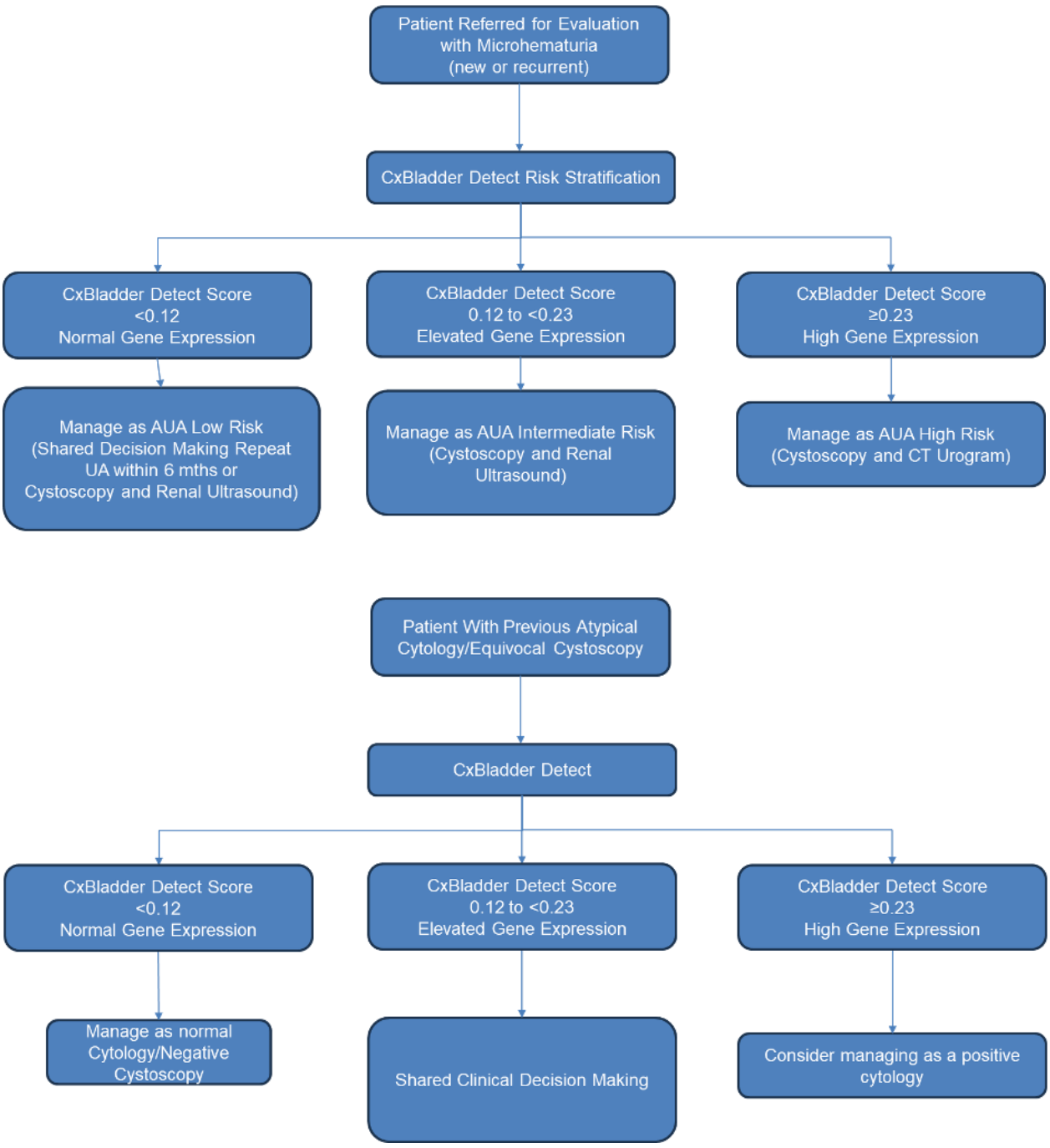
These patients are risk stratified by AUA guidelines into low, intermediate, and high risk microhematuria based on clinical and demographic factors with the approximate percentages being 5%, 12%, and 83% respectively(6).
Studies have shown that most of these patients do not have UC, with prevalence data showing approximately 5% of patients having UC(6).
The current standard of care for those patients is dependent on their risk stratification, where only low risk patients are counseled to return in 6-8 months for another urine analysis (UA) to determine if they need a full workup. Intermediate and high-risk patients on the other hand are provided a full workup, including a cystoscopy, to assess the bladder and CT (Computerized Tomography) urography to assess the upper tract. The risks associated with this standard of care are those associated with any invasive procedure including infections, urethral damage, and any allergic reactions to contrast agents used for CT urography to name a few.

The clinical utility of Detect is driven by the high negative predictive value that identifies the patients that present with microhematuria that are at significantly lower risk of currently having UC so that they can be given lower intensity diagnostic evaluations. The value to the Medicare population of adopting Detect prior to cystoscopy is the reduction of unnecessary cystoscopy and imaging procedures for patients who do not need it, while simultaneously improving the yield of cancer diagnoses within the patients that do receive the full workup.

Proposed Eligibility Criteria for Cxbladder Detect

- Microhematuria (MH) patients referred to the urology office for evaluation.
    - MH is defined as ≥3 RBC/HPF with no visible blood in urine.
    - Gross hematuria for adjudication of diagnostic dilemmas.
- Non-malignant or gynecologic causes ruled out by urologist prior to ordering the test.
    - UTI (urinary tract infections), kidney stones, etc..., ruled out.

The diagram below illustrates the two clinical pathways for Cxbladder Detect:



Conclusion

Cxbladder Detect should be a covered benefit for Medicare patients under the LCD because:

- It has demonstrated analytical validity, clinical validity, and clinical utility in published studies ***(see appendix for specific studies)***.
- The current standard of care drives significant overuse of diagnostic procedures, specifically invasive and unpleasant cystoscopy. For lower risk patients, this has a

disproportionate impact on the elderly given the higher rates of complications in patients with multiple co-morbidities.

The clinical value of expanded use of tests with high negative predictive value also benefits patients as it reduces the financial burden on the health care system by removing patients from unnecessary procedures with no impact on patient outcomes.

Cxbladder Triage

Clinical Scenario and Patient Population

Triage is indicated to risk stratify and identify lower-risk hematuria patients to reduce the burden of unnecessary investigations. It is intended for use by primary care physicians or at the urology office to prioritize patients and manage unnecessary referrals for more invasive evaluation at the urology office.
The clinical utility of Triage is to identify the patients that present with microhematuria that have significantly lower risk of currently having UC so that they can be managed according to the low risk AUA guidelines recommendation rather than given a full workup that is unnecessary for those patients.
The value to the Medicare population of adopting Triage prior to cystoscopy is reduction of unnecessary cystoscopy and imaging procedures for patients at lower risk, while simultaneously improving the yield of cancer diagnoses within the patients that do receive the full workup.

Proposed Eligibility Criteria for Cxbladder Triage

- Microhematuria (MH) patients at the Primary care office, or low risk patients referred to the urology office.
- MH is defined as ≥3- 25 RBC/HPF with no previous incidence of gross hematuria.
- Non-malignant or gynecologic causes ruled out by urologist prior to ordering the test.
    - UTI, kidney stones, etc..., ruled out.

Conclusion
Cxbladder Triage should be a covered benefit for Medicare patients under the LCD because:

- It has demonstrated analytical validity, clinical validity, and clinical in published studies ***(see appendix for specific studies).***
- The current standard of care drives significant overuse of diagnostic procedures, specifically invasive and unpleasant cystoscopy. For lower risk patients, this has a disproportionate impact on elderly patients given the higher rates of complications in patients with multiple co-morbidities.

The clinical value of expanded use of tests with high negative predictive value also benefits patients as it reduces the financial burden on the health care system by removing patients from unnecessary procedures with no impact on patient outcomes.

Cxbladder Monitor

Clinical Scenario and Patient Population

The Cxbladder Monitor test is intended for use in patients with prior diagnosis of NMIBC that are on a surveillance protocol for follow up for recurrence of disease. The test is intended for use starting at 9 months post diagnosis of either primary or recurrent disease with no recurrence in between. The test should be used in an alternating fashion with cystoscopy to reduce the diagnostic burden on these patients. Patients with a negative test (NPV (Negative Predictive Value) 97%) can defer the cystoscopy to the next scheduled surveillance visit with no impact in identifying recurrence. Those patients with a positive test should continue with the normal surveillance protocol.

There are approximately 800,000 patients that are seen annually for UC recurrence in the U.S. Bladder cancer has a high rate of recurrence with a 1-year recurrence rate of 15-61%, and a 5-year recurrence rate of 31-78%(7). Therefore, patients are subjected to a frequent and intensive monitoring schedule. These patients generally follow a surveillance protocol that calls for cystoscopy at regular intervals depending on the risk classification of those patients (AUA guidelines table below). High and intermediate risk patients are followed up every 3 months for the first two years of surveillance, every 6 months for years 3-4, then annually afterwards with no set limit to stop surveillance. Low risk patients are recommended for follow up at 3 months post diagnosis, 9-12 months after that, then annually for the next 5 years. It is important to note that all these recommendations are restarted if recurrence of disease occurs, and cystectomy is not recommended.

The risks associated with this standard of care are similar to those described above for the hematuria evaluation, with the added risk of repeat incidence of the same problems over a prolonged period of surveillance.

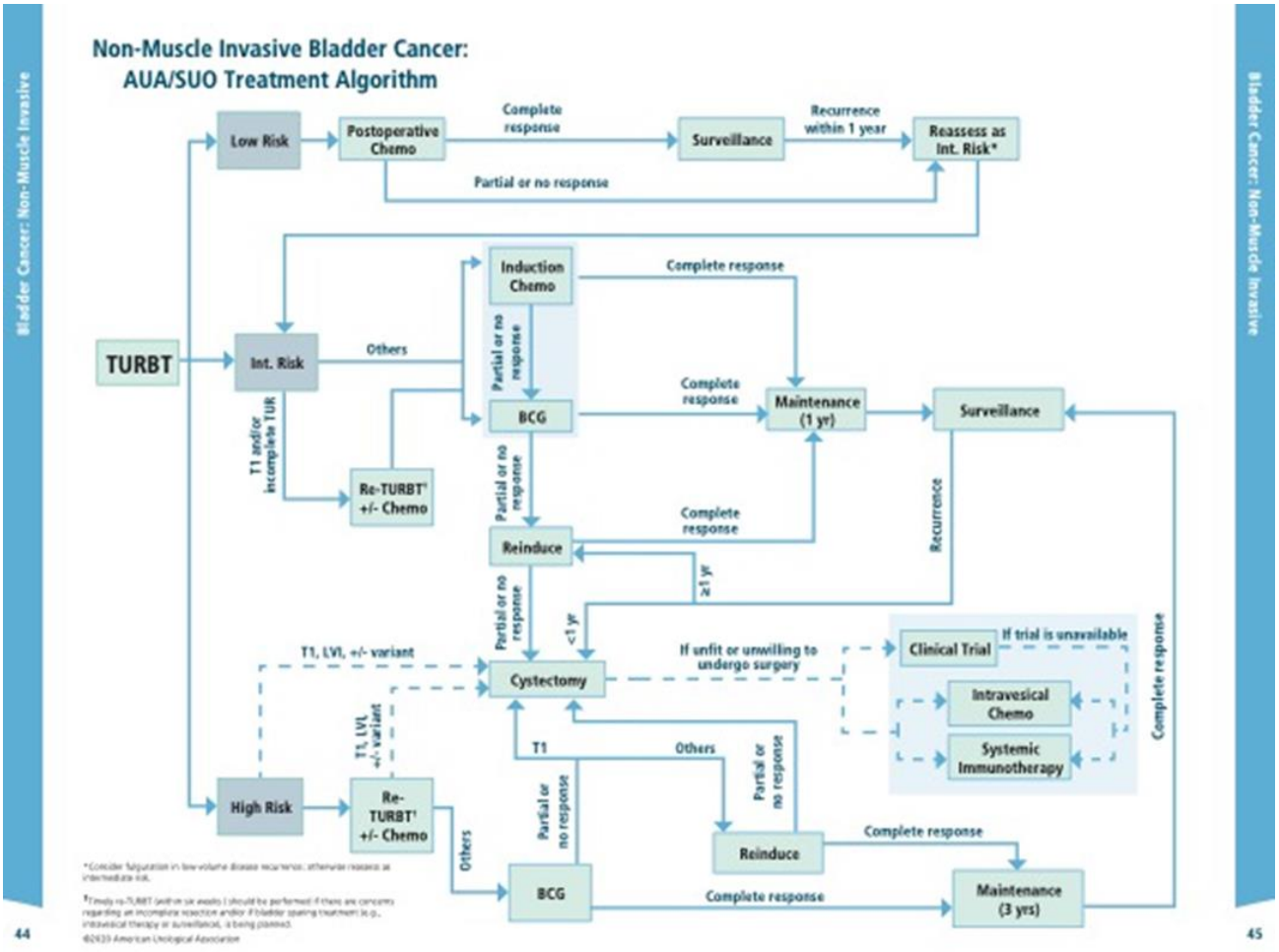
The clinical utility of Monitor is to identify the patients that are at a low enough risk of recurrence so that they can safely alternate cystoscopy with Cxbladder Monitor test within the timeframes of standard of care.

The value to the Medicare population of alternating Monitor with cystoscopy during standard surveillance protocols is to reduce the burden of invasive procedures on the patient and health care system and improve patient compliance with the surveillance protocols. If low risk patients can safely defer the surveillance visit and alternate with the test, the higher risk patients will have priority at the urology office and early detection of any recurrence will be standard of care.

The chart below is AUA guideline for NMIBC.

Table 2. *AUA risk stratification for non-muscle invasive bladder cancer*

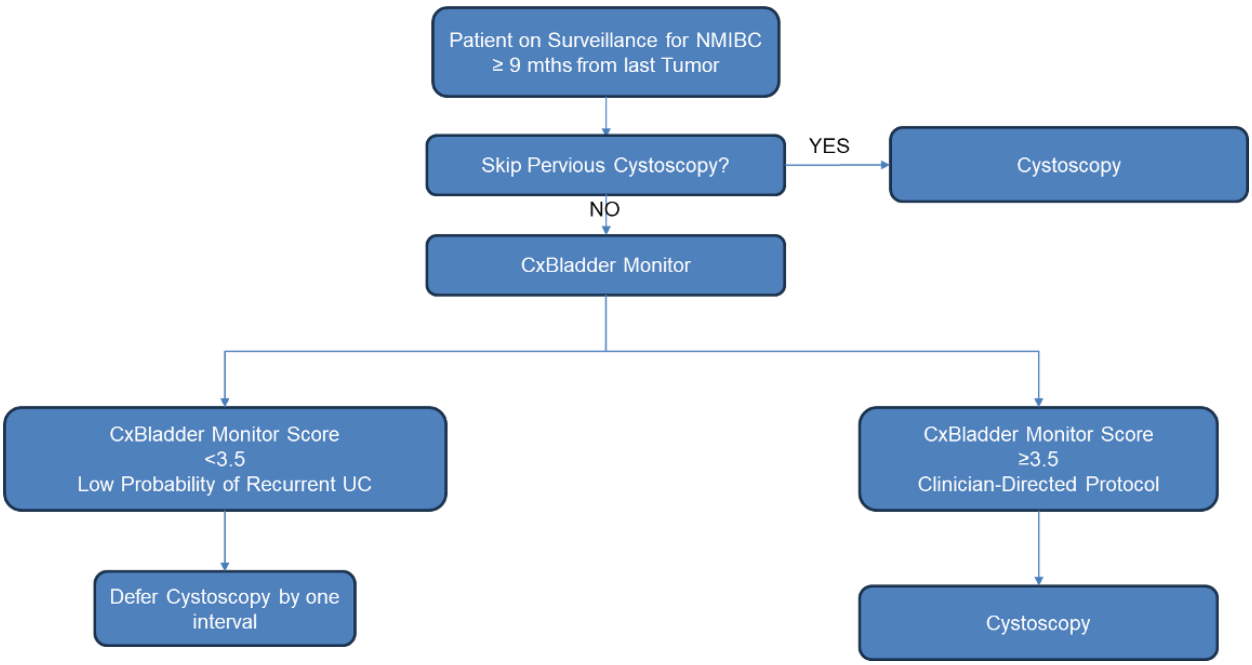
Low Risk	Intermediate Risk	High Risk
Low grade solitary Ta ≤ 3 cm Papillary urothelial neoplasm of low malignant potential	Recurrence within 1 year, low grade Ta Solitary low grade Ta >3 cm Low grade Ta, multifocal High grade Ta, ≤3 cm Low grade T1	High grade T1 Any recurrent, high grade Ta High grade Ta, >3 cm (or multifocal) Any CIS Any BCG failure in high grade case Any variant histology Any LVI Any high grade prostatic urethral involvement



Proposed eligibility criteria for Cxbladder Monitor test:

- Patients with previously diagnosed urothelial cancer (primary or recurrent, any risk classification) have at least 9 months of recurrence free follow up. Those patients may alternate the Cxbladder Monitor test with regular cystoscopy and can prolong the duration between cystoscopies based on a negative Monitor test.

- Low risk patients who have no recurrence for 3 years. Those patients can be setup to receive a Cxbladder Monitor test every 6-12 months in place of regular cystoscopy. Positive Monitor test should be referred for cystoscopy.
- Intermediate and High-risk patients who have no recurrence for 5 years. Those patients can be setup to receive a Cxbladder Monitor test every 6 months in place of regular cystoscopy. Positive Monitor test should be referred for cystoscopy.



Conclusion

Cxbladder Monitor should be a covered benefit for Medicare patients under the LCD because.

- It has demonstrated analytical validity, clinical validity, and clinical utility in published studies (see Appendix for specific studies)
- The current standard of care drives significant overuse of diagnostic procedures, specifically invasive and unpleasant cystoscopy. For lower risk patients the overuse of invasive procedures has a disproportionate impact on elderly patients given the higher rates of complications in patients with multiple co-morbidities. These benefits are exaggerated in the surveillance population due to the repetitive nature of surveillance. The more patients can avoid unnecessary procedures, the better quality of life for those patients.

- The clinical value of expanded use of tests with high negative predictive value also benefits patients as it reduces the financial burden on the health care system by removing patients from unnecessary procedures with no impact on patient outcomes(8).

Support for Request #2

Request 2a -The focus of this LCD is on genetic tests that are performed after a biopsy-proven (histologic or cytologic) diagnosis of cancer or substantiated suspicion of cancer based on histology or urine cytology. As Cxbladder Triage and Cxbladder Detect are indicated for patients with hematuria prior to a diagnosis of cancer. Hematuria appears not to meet the requirements for suspicion of cancer set forth in the draft LCD, yet hematuria is a key factor in determining if a full diagnostic workup for urothelial cancer is warranted according to the urology community standard of care. If in the final LCD hematuria is not recognized as a substantiated suspicion of bladder cancer, **then Cxbladder Triage and Cxbladder Detect tests as well as any other tests performed for the evaluation of hematuria where a diagnosis of bladder cancer has not yet been substantiated based upon histologic or cytology findings should not fall under such LCD.** Neither a coverage determination nor a non-coverage determination is appropriate under this LCD for Cxbladder Triage and Detect because it is not the intended purpose of these tests to be ordered in patients for whom the diagnosis of bladder cancer has already been made. It is also worth noting that the NCCN (National Comprehensive Cancer Network) guidelines, which are used as one of the criteria for coverage in this LCD, do not address hematuria evaluation at all—these guidelines focus on the evaluation and management of patients with bladder cancer. As such, inclusion of tests for the evaluation of hematuria in this proposed LCD is not appropriate given that three knowledge bases referenced in the LCD would not include RNA based test indicated for hematuria evaluation.

Request 2b – If Novitas and First Coast concur that Cxbladder Triage and Detect should not be included in the LCD, **we respectfully request that Medicare coverage continue as it has been since July 2020**. This coverage should include a specific reference in LCA 58917 or other appropriate articles providing clarity to clinicians and Medicare Advantage payers on the positive coverage of Triage and Detect. It would cause significant confusion in the marketplace if Triage and Detect were removed from the current LCD and not referenced in another Novitas document. Any such confusion would be detrimental to Medicare beneficiaries given that these tests have been consistently covered for multiple years.

Request 2c - If Novitas and First Coast determine that Cxbladder Triage and Detect's use falls outside of the current LCD, it will be important to develop an LCD for urinary biomarkers for the evaluation and management of patients with hematuria as that is a critical part of the bladder cancer diagnostic process. **It is our recommendation that a new LCD should be developed with the support of convening a Contractor Advisory Council to ensure that input from clinician experts treating Medicare beneficiaries is part of the LCD development**.

Request 3 - Cxbladder Resolve, Cxbladder Enhanced Triage, and Cxbladder Enhanced Detect are not currently clinically available in the U.S. These tests have not been fully validated at this point. Therefore, we believe it is premature and inappropriate to include a detailed evidentiary review of these tests resulting in preemptive non-coverage in the draft LCD and that **these tests should be excluded from the LCD**.

The Novitas/FCSO review included evaluation of Cxbladder Resolve and the "enhanced" Cxbladder tests that include single nucleotide polymorphisms. These are tests under development and have not been validated for clinical use at this time. None of these are commercially available in the US. Indeed, large prospective multicenter clinical trials are currently underway (Appendix) to provide evidence of clinical validity and clinical utility of those tests and their clinical applications. When these tests have been fully validated and are being offered for clinical use, then it may be appropriate for Novitas/FSCO to evaluate the evidence and determine if these tests meet the requirements to be medically reasonable and necessary.

We thus believe the criticism of these tests as having insufficient evidence is premature and should not influence the assessment for coverage of the commercially available tests, specifically, Triage, Detect, and Monitor.

Request Summary

We respectfully request that Novitas re-evaluate the important clinical role that Cxbladder Triage, Detect, and Monitor have in the hematuria evaluation and recurrence monitoring pathways for bladder cancer. We believe that our requests are supported by the clinical evidence and the needs of clinicians and patients. Our requests are consistent with the LCD and supported by the primary clinical organizations and societies within the urology community.

COMMENTS ON EVIDENTIARY REVIEW OF CXBLADDER PUBLISHED LITERATURE:

Detailed comments responding to the evidentiary review in the draft LCD are presented below for Cxbladder Triage, Detect, and Monitor. These comments form a significant portion of the rationale for inclusion of the Cxbladder tests as covered test in the LCD as the review of the published literature included several incorrect assumptions and misunderstandings. Taken as a whole, the comments below show that the published data on the Cxbladder products surpasses the level of evidence required for Medicare coverage as medically reasonable and necessary.

1) THE HOLYOAKE STUDY IS A BIOMARKER DEVELOPMENT STUDY, NOT A VALIDATION STUDY

Novitas/FSCO considered the Holyoake study to be the initial development study for Cxbladder Triage and Detect and conclude that the study does not show that the tests distinguish among various types of cancers. This assessment is then used as the basis for determining that none of the Cxbladder tests meet the reasonable and necessary coverage criteria because they were built on a faulty foundation. However, the evidentiary review does not reflect the fact that the Holyoake study was designed to identify potentially relevant biomarkers to help inform development of the Cxbladder assays – it was not designed as an initial study supporting the validity of the assays themselves.

In their review, Novitas has claimed that our initial development study(9) was flawed in its design:

> "This means that a well-designed test will be able to not only discriminate between cancer and normal tissue, but also between different types of malignancy."(10) (p.32)

In another portion of the review Novitas states:

> "One very notable gap included a lack of details or definition for non-urothelial cancers, of which many would feed into the urinary system, including prostate cancers, renal cancers, and metastatic or locally invasive cancers from other organs(10)."
>
> *** * ***
>
> "This first paper from 2012 also does not sufficiently address Cxbladder' s ability to distinguish between urothelial carcinoma and other malignancies, which is of particular relevance when a majority of the patient population were male (78%) with a median patient age of 64 years and thus, with higher risk of prostate carcinoma."(10) (p.33)

The Holyoake (2008)(9) paper is fundamentally misinterpreted to be a "development study" for clinical assays. This study was a "biomarker discovery" study aimed to identify which biomarkers play a role in various cancers that could subsequently inform the development of the Cxbladder assays which then would be assessed in future studies to determine the analytical validity, clinical validity, and clinical utility of the specific assays developed. Concluding that the Cxbladder assays do not meet criteria for reasonable and necessary based on a biomarker discovery study is not appropriate because such a study does not evaluate any specific test nor is it intended to provide evidence of the analytical validity, clinical validity, or clinical utility of any test developed comprising any biomarkers discovered in such a study.

Furthermore, these excerpts above from the review show an unfamiliarity with the established clinical pathway for the management of patients presenting with hematuria that supports the clinical utility and intended use of our tests. The Cxbladder Triage and Detect tests were developed to determine if a urine-based test can distinguish between presence or absence of urothelial cancer with the stated goal of reducing the burden of unnecessary invasive procedures (cystoscopy, CT-Urogram, ureteroscopy) for ***patients presenting with hematuria***.

The purpose of the development study was ***not*** to develop a test to distinguish between urothelial carcinoma and other types of cancer. We also point out that prostate cancer does not typically

present with hematuria and neither prostate nor renal cancers are diagnosed by cystoscopy. Both of these cancers have their own presentation symptoms and signs and have pre-defined diagnostic pathways that best represent how they are diagnosed. The Cxbladder tests were developed specifically to address the clinical management of patients presenting with hematuria in the absence of other known benign or malignant disease where there is suspicion of bladder cancer and more invasive evaluation for bladder cancer (i.e., cystoscopy) is being considered. The Cxbladder tests were not designed to address other clinical questions, such as patients presenting with an elevated PSA (Prostate Specific Antigen) being evaluated for prostate cancer or patients presenting with a renal mass being evaluated for renal cell carcinoma. We have heard from the urology community that use our tests are helpful in the clinical evaluation of patients with hematuria and monitoring for recurrence as these tests address the likelihood that the patient has bladder cancer, which is the key question in the evaluation of these patients. We have been told by several urology stakeholders that they plan to comment on the draft LCD reinforcing these points.

Our tests were developed for the purpose of reducing the investigative burden on patients with substantiated suspicion of disease by standard of care, i.e., those patients presenting with hematuria, and for these purposes, Cxbladder tests perform exactly as intended. The tests should remain covered accordingly.

2) THE DRAFT LCD DOES NOT REFLECT THE ESTABLISHED CLINICAL PATHWAYS IN WHICH THE CXBLADDER TESTS ARE USED

Novitas appears to have misunderstood the clinical value and benefits of our tests in their review of literature. In our response above, we have included both written and graphic descriptions of the clinical scenarios in which the Cxbladder products are used to benefit patients. The written descriptions are copied below for your reference. Pacific Edge would strongly support assembling a Clinical Advisory Committee (CAC) comprising experts in the management of patients presenting with hematuria to inform the development of any coverage policy addressing the use of urinary biomarkers in the management of hematuria. Pacific Edge is willing and ready to collaborate with the Novitas/FCSO medical team at any time to provide appropriate additional information on the utility and benefit of the Cxbladder Triage and Detect tests in the Medicare population.

We provide here the intended clinical pathways for the Cxbladder Triage and Detect tests together with the data supporting their Analytical Validation (AV), Clinical Validation (CV), and Clinical Utility (CU).

a- **Cxbladder Triage Test:** Intended to risk stratify and identify lower risk microhematuria patients to reduce the burden of unnecessary investigations. It is intended for use by primary care physicians or advanced practice clinicians (e.g., NPs or PAs) to prioritize

patients and manage unnecessary referrals for more invasive evaluation at the urology office.

b- **Cxbladder Detect Test:** Intended for use in urology practices with any microhematuria patient to risk stratify those patients into low, intermediate, and high risk of bladder cancer. It is intended to reduce the burden of investigations for the low and intermediate risk patients after shared decision making with the patient and prioritize those with high risk for full investigation. The test can also be used to adjudicate diagnostic dilemmas when cytology is equivocal, or cystoscopy is un-informative in both microscopic and gross hematuria patients.

c- **The Cxbladder Monitor Test:** Intended for use in patients with prior diagnosis of NMIBC that are on a surveillance protocol for follow up for recurrence of disease. The test is intended for use starting at 9 months post diagnosis of either primary or recurrent disease with no recurrence in between. The test should be used in an alternating fashion with cystoscopy to reduce the burden on these patients. Patients with a negative test (NPV 97%) can defer the cystoscopy to the next scheduled surveillance visit, those with a positive test, should be referred for cystoscopy.

The Appendix summarizes the studies used to provide the validation and utility of these tests in these specific patients.

3) Patient demographics

The Novitas/FCSO review included a concern that there is a male bias in the studies supporting the use of the Cxbladder Triage and Detect tests. We disagree with this critique of the evidence base. Bladder cancer has a much higher incidence in men than women with diagnoses in the USA 3-4x more frequently in men than women (https://www.cancer.org/cancer/types/bladder-cancer.html). If the studies had an equal balance of men and women, then the studies would not be representative of the target population.

4) Discrimination between Urothelial Cancer and Other Cancer Types

As discussed above, the LCD does not reflect the intended use and clinical value of Cxbladder Triage and Detect. These tests are not designed to distinguish between multiple cancers or serve as multi-cancer early detection (MCED) tests trying to answer whether the patient has a cancer anywhere. These are risk stratification tests that specifically attempt to reduce the use of unnecessary, invasive, and potentially harmful investigations (cystoscopy, CT Urogram, ureteroscopy) in populations with substantiated suspicion of urothelial cancer(11). In the case of Cxbladder Triage this includes patients presenting with hematuria that have a high chance of normal evaluation, but for whom American Urological Association (AUA) guidelines advocate more invasive investigations. Prostate cancer is not diagnosed through cystoscopy or CT Urogram

and rarely presents with hematuria unless very advanced. The fundamental question answered by Cxbladder tests is "can a negative test identify a patient with a low enough risk of the presence of urothelial cancer that can avoid further unnecessary evaluation" and for that question the test has performance characteristics that provide high clinical value. In addition, patients presenting with other types of urogenital cancers have a unique set of symptoms and signs that are specific to those cancers. Urological societies have separate diagnostic pathways in their respective guidelines for evaluation of those patients. In the case of prostate cancer, initial workup depends on an elevated level of PSA and not hematuria. If the PSA level is elevated, patients can undergo multiple imaging studies (U/S, MRI) prior to a decision for biopsy which would be the only true diagnostic step for prostate cancer(12). In the case of renal cell carcinoma, patients will usually present with dull aching pain in their loin with or without a palpable mass. These patients are referred to CT scan to identify the lesion and are managed completely differently than UC in the upper tract(13).

5) Criticism for lack of studies done independently of Pacific Edge

We do not believe that company sponsorship of clinical trials is a valid criticism of our studies as that practice is common in the industry. The Novitas review mentions that part of the problem is lack of confidence in Pacific Edge data since many of the studies reviewed were either funded or performed by Pacific Edge Limited. Most new drugs, biologicals, devices, and diagnostics have the development studies funded by the sponsor because there is no other entity who is likely to conduct the necessary studies to determine the safety/effectiveness (drug/biological/device) or AV/CV/CU (diagnostic). If these studies have appropriate trial designs for the intended uses and the data is analyzed consistent with prospectively established analysis plans, then these studies can be considered appropriate for coverage review. It is imperative for our company and others to maintain the highest quality of evidence by supporting such studies to ensure that patients and physicians have access to high quality data. We would also maintain that although many of the studies were funded by Pacific Edge, there were many well respected thought leaders in the field that participated in the design and execution of these studies to prove the value of these tests for their patients, including Medicare patients. All published data was subject to peer review and external editor questions that is designed to confirm the validity of the data. Finally, Novitas does not mention in its review that several of our recent, most powerful real-world evidence for the clinical utility of Cxbladder tests were done with no company support. Specifically, for Cxbladder Triage in Davidson et al (2019 and 2021)(14, 15) and for Cxbladder Monitor in Li et al (2023)(8) were all conducted completed independently of Pacific Edge with no financial support and no provision of testing resources by the company.

6) Short follow up

Another criticism of the data supporting the use of Cxbladder tests was the short follow up time. The suite of Cxbladder tests address a relative short-term clinical question—when patients present with hematuria, can the tests identify patients with such low risk of bladder cancer that more invasive testing can be avoided at that time. The Cxbladder tests are not intended for

treatment predictive or prognostic uses. Foundationally, this means that they are designed to inform an **immediate decision** regarding whether invasive procedures, e.g., cystoscopy, CT Urogram, ureteroscopy that are mandated by the guidelines can be safely omitted or not for a defined patient population. As they are neither predictive nor prognostic tests, they are not designed to assess the risk of developing cancers in the future. **Therefore, the follow-up included in each study is appropriate for the intended clinical use.**

In the case of Triage and Detect tests, the intended clinical use is for patients presenting with primary or recurrent microhematuria. In the clinical application of these tests, the risk stratification can either determine that the patient has a "Low Probability of UC" (Cxbladder Triage report; see Appendix) or "Normal gene expression score" (Cxbladder Detect report; see Appendix). In each case such a report enables the physician and patient to safely *defer* a full evaluation for Urothelial cancer if they choose to do so. A "not negative" Cxbladder Triage result of "Standard clinical workup" is an indication to follow standard of care. A Cxbladder Detect result of "high gene expression score" is a higher likelihood of disease with a recommendation to follow AUA guidelines evaluation steps and initiate a full workup. In AUA guidelines, the recommendation of low-risk patients with hematuria would be to bring them back at ***6-9 months*** for a repeat urine analysis to assess if the hematuria persists. This means that the follow up provided in our studies is appropriate for those patients.

7) Criticism of low positive predictive value

In the draft LCD, Novitas/FCSO raise concerns about the PPV (Positive Predictive Value) for the Cxbladder Triage and Detect tests. This concern reflects a misunderstanding of the intended use of our tests as a rule out bladder cancer tests. The draft LCD states,

> "These values are significant in that false test results, particularly false positives, can lead to patient anxiety and distress among other procedural issues related to follow up for an inaccurate result." (10) [(p.35)]

As explained previously in the document, these tests were optimized for high sensitivity and high NPV intended to help identify low risk patients that can be safely ruled out from a diagnosis of bladder cancer in order to reduce the burden of unnecessary procedures on patients. The clinical value of our tests in the hematuria evaluation population (Triage and Detect) is to identify those patients that have the low likelihood of urothelial cancer and thus can defer further unnecessary workup. If any of our tests are ***positive***, the patient is to continue with the normal guidelines recommended investigations for their hematuria. In the absence of such high NPV tests, all patients presenting with hematuria would be subject to a full invasive workup even though the prevalence of UC is low. Thus, if the value of the test is fully understood, no additional burden of testing or morbidity from a positive test will be encountered.

Furthermore, all our marketing materials along with our scientific medical exchanges explain clearly to physicians that point and provide recommended explanations for patients on the value of the negative to reduce the anxiety that may be caused by a non-negative result. In addition,

the evidentiary review included in the draft LCD did not include the fact that the guideline directed standard of care has many more patients receiving a full workup, with many of those patients being disease free, which translates to a much higher rate of anxiety and concern on the part of the patients, as well as an increased financial burden on the system and patients alike(16).

8) Lack of data on patients with Inflammatory processes

The LCD review also notes that our studies excluded patients with inflammatory processes (exclusion of active UTI, UT manipulations, etc.). We find this to be a lack of understanding of how our test is developed and how it is being utilized by over 4,000 physicians in the marketplace. Our Cxbladder Triage and Detect tests are indicated for patients that present with hematuria where inflammatory or infectious causes have been ruled out and the clinical concern is focused on the diagnosis of bladder cancer and the extensive evaluation required.

Cases with high inflammation can negatively impact the test results and may cause an unwarranted false positive or false negative and therefore we exclude patients with conditions that can be associated with inflammation (see below). We attempted to reduce the impact of high inflammation samples by adding the 5th gene (CXCR2) which is an inflammatory gene to reduce that risk(2). Although the inclusion of the 5th gene did reduce the impact of inflammation considerably(2), along with many other minor changes we had made to the process, it did not eliminate it completely. Therefore, in accordance with our commitment to the highest standards and to maintain the best outcomes for our patients, we do not accept any commercial samples from patients with any of these issues. In fact, here are our exclusions for our commercial samples:

Exclusions for COLLECTION PROCESS: (see appendix)

- Visible blood
- Dip sticks cannot be left in urine cup before transferring to Cxbladder tube.

WAIT 6 WEEKS FROM:

- BCG (Bacillus Calmette Guérin) therapy
- Mitomycin therapy
- Radiation therapy
- Any bladder manipulation

WAIT 2 WEEKS FROM:

- Catheterization
- Cystoscopy
- Bladder infection or UTI (should finish antibiotics and wait 2 weeks)
- Trace leukocytes

Thus, we are consistent in our inclusion and exclusion criteria for both our studies and our commercial usage. We excluded those patients from our studies and from our commercial acceptance criteria to provide the highest level of service for the other patients that would benefit from our tests.

SUMMARY OF COMMENTS ON EVIDENTIARY REVIEW

The comments above represent a response to the criticisms Novitas found in their evidentiary review of the Cxbladder data. We believe that Novitas misinterpreted important aspects of our published literature which led to incorrect conclusions about the strength of our data in the clinical care of patients at risk or with confirmed bladder cancer. We respectfully suggest that the criticisms should be re-examined in light of our comments and should not invalidate the clinical data that has been developed for Cxbladder tests.

Appendix - Summary of clinical evidence

		Study	Pop. Type	Sensitivity (Sn)	NPV	Specificity (Sp)	PPV	Comment
Triage	**AV**	Kavalieris et al., 2015	MH + GH*	95%	98%	45%	--	Sn, Sp, NPV values when test-negative rate is 40%
	CV	Davidson et al., 2019	MH + GH*	95.5% (**1**)	98.6% (**1**)	34.3%	--	GH only: **Sn** (95.1%), **NPV** (98%), **Sp** (32.8%); MH only: **Sn** (100%), **NPV** (100%), **Sp** (42.6%)
		Konety et al., 2019	(**2**)	(**3**)	--	--	--	Cxbladder (**3**) correctly adjudicated all UC confirmed patients (*n*=26) with atypical urine cytology results (*n*=153, **4**); test-negative rate of 35%.
		Raman et al., 2021	MH + GH* (**5**)	92.6%	99.6%	--	--	Test-negative rate of 52%.
		Lotan et al., 2023	MH + GH*	89%	99%	63%	16%	Pooled data from US and Singapore cohorts (*n*=804), test-negative rate of 59%.
	CU	Davidson et al., 2020	MH + GH*	89.4% (**6**)	98.9% (**6**)	59% (**6**)	--	39% of patients testing negative for CxbT & imaging did not get cystoscopy & were managed at primary care (**7**), test-negative rate of 53%.
		STRATA (unpublished) (**8**)	MH + GH*	--	--	--	--	*Study in progress*

Detect	**AV**	O'Sullivan et al., 2012	GH*	81.8%	97%	85.1%	--	0.12 test score cut-off - CxbD detected 97% of HG tumors & 100% of >T1 tumors
				77%	96%	94%	--	0.23 test score cut-off – PPV 68%; unpublished data after paper O'Sullivan paper publication
	CV	Lotan et al., 2023	MH + GH*	74%	97%	82%	25%	Pooled data from US and Singapore cohorts (*n*=804), test-negative rate of 78%.
		DRIVE (unpublished) (**1**)	MH + GH*	--	--	--	--	*Study in progress*

		Study	Pop. Type	Sensitivity (Sn)	NPV	Specificity (Sp)	PPV	Comment
Monitor	**AV**	Kavalieris et al., 2017	(**1**)	88% (**2**)	97% (**2**)	--	--	(**3**), test-negative rate of 34%.
	CV	Konety et al., 2019	(**4**)	100% (**5**)	--	--	--	Cxbladder (**5**) correctly adjudicated all UC confirmed (*n*=26) with atypical urine cytology results (*n*=153, **6**), test-negative rate of 35%.
	CU	Koya et al., 2020	(**7**)	--	--	--	--	Integration of CxbM into surveillance schedule could reduce annual cystoscopies by 39% (**8, 9, 10,11**)
		Li et al, 2023	(**12**)	100%	100%	78%	33%	A prospective multi-institutional study of CxbM to reduce surveillance frequency during the coronavirus pandemic, test-negative rate of 73%.
		Sfakianos et al (unpublished)	(**13**)	--	--	--	--	A retrospective audit of clinical use of CxbM with patients undergoing surveillance cystoscopy

	References
Triage	Davidson et al., (2019). Inclusion of a molecular marker of bladder cancer in a clinical pathway for investigation of haematuria may reduce the need for cystoscopy. NZ Med J, 132(1497), 55-64.
	Davidson et al., (2020). Assessment of a clinical pathway for investigation of haematuria that reduces the need for cystoscopy. NZ Med J, 133(1527), 71-82.
	Kavalieris et al., (2015). A segregation index combining phenotypic (clinical characteristics) and genotypic (gene expression) biomarkers from a urine sample to triage out patients presenting with hematuria who have a low probability of urothelial carcinoma. BMC urology, 15(1), 1-12.
	Konety et al., (2019). Evaluation of Cxbladder and adjudication of atypical cytology and equivocal cystoscopy. European urology, 76(2), 238-243.
	Raman et al., (2021). The diagnostic performance of Cxbladder Resolve, alone and in combination with other Cxbladder tests, in the identification and priority evaluation of patients at risk for urothelial carcinoma. The Journal of Urology, 206(6), 1380-1389.
	Lotan et al., (2023). Urinary Analysis of FGFR3 and TERT Gene Mutations Enhances Performance of Cxbladder Tests and Improves Patient Risk Stratification. The Journal of Urology, 209(4), 762-772.

Detect	Lotan et al., (2023). Urinary Analysis of FGFR3 and TERT Gene Mutations Enhances Performance of Cxbladder Tests and Improves Patient Risk Stratification. The Journal of Urology, 209(4), 762-772.
	O'Sullivan et al., (2012). A multigene urine test for the detection and stratification of bladder cancer in patients presenting with hematuria. The Journal of Urology, 188(3), 741-747.

	References
Monitor	Kavalieris et al., (2017). Performance characteristics of a multigene urine biomarker test for monitoring for recurrent urothelial carcinoma in a multicenter study. *The Journal of Urology*, *197*(6), 1419-1426.
	Konety et al., (2019). Evaluation of Cxbladder and adjudication of atypical cytology and equivocal cystoscopy. European urology, 76(2), 238-243.
	Koya et al., (2020). An evaluation of the real world use and clinical utility of the Cxbladder Monitor assay in the follow-up of patients previously treated for bladder cancer. *BMC urology*, *20*(1), 1-9.
	Li et al., (2023). Cxbladder Monitor testing to reduce cystoscopy frequency in patients with bladder cancer. *Urologic Oncology: Seminars and Original Investigations, 41(7), 326.e1-326.e8.*
	Lotan et al., (2017). Clinical comparison of noninvasive urine tests for ruling out recurrent urothelial carcinoma. *Urologic Oncology: Seminars and Original Investigations*, 35 (8), 531-539.
	Sfakianos et al, unpublished

Appendix: – Cxbladder Test reports

Cxbladder™ DETECT

PACIFIC EDGE DIAGNOSTICS USA LTD

Patient Name:
Patient Date of Birth:
Patient Sex:
Medical Record #:

Sample ID:
Alternate ID:
Specimen Source:
Specimen Collection Date:
Specimen Receipt Date:
Specimen Report Date:
Report Status:
ICD-10 Code:
Ordering Clinician:

Test Result: Cxbladder Detect score

NORMAL Gene Expression Score	ELEVATED Gene Expression Score	HIGH Gene Expression Score

0.00 0.12 0.23 1.00

Negative UC Detection — Positive UC Detection

Comments:

Results Interpretation:

The Cxbladder Detect test was developed and validated on 476 patients recruited under an international multicenter clinical study of patients presenting with macro-hematuria. The patients were allocated to a group of 317 for development and 159 for validation. 100% of T1, T2, and T3 tumors were placed in the 'HIGH Gene Expression Score' region and 67% of the Ta tumors in the 'ELEVATED or HIGH Gene Expression Score' regions. A Cxbladder Detect score of greater than or equal to 0.12 has a sensitivity of 82% and a specificity of 82%. Cxbladder Detect is a development from the clinical study published in J. Urology (2012) 188:741-747.

NORMAL Gene Expression Score < 0.12	A score of < 0.12 has an NPV of 97%. High probability of NO urothelial carcinoma.
ELEVATED Gene Expression Score 0.12 ≤ score < 0.23	A score of 0.12 ≤ score < 0.23 has an NPV of 94%. Low probability of urothelial carcinoma, however a change in the pattern of gene expression of the biomarkers from what is normal suggests further clinical evaluation.
HIGH Gene Expression Score ≥ 0.23	A score of ≥ 0.23 has a specificity of 94% and a PPV of 68%. High probability of urothelial carcinoma.

Assay Description:

Cxbladder Detect is a quantitative test that uses reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) to measure five mRNA biomarkers (CDK1, MDK, IGFBP5, HOXA13 and CXCR2) in a small sample of a patient's urine. An algorithm is applied to the measured quantities of these biomarkers to calculate a composite Cxbladder Detect score ranging from 0.00 to 1.00. Cxbladder Detect test results are intended to aid in the detection of urothelial carcinoma (UC) when used in conjunction with standard clinical assessment.

Reviewed By: Thomas P. Nifong, MD (electronically signed)
Laboratory Director, Pacific Edge Diagnostics USA Ltd

Disclaimer: This is a high complexity clinical test developed by and its performance characteristics determined by Pacific Edge Ltd. This test is for clinical purposes and is intended to aid the clinician in determining the likelihood of the presence of urothelial carcinoma (UC). It should not be regarded as investigational or for research only. This test has not been approved by the US Food and Drug Administration (FDA) and such approval is not required. The laboratory is regulated under Clinical Laboratory Improvement Amendments of 1988 (CLIA).

Pacific Edge Diagnostics USA Ltd, Hershey Center for Applied Research, 1214 Research Boulevard, Suite 2000, Hummelstown, PA 17036
Tel: 1-855-CXBLADR (1-855-292-5237) Fax: (717) 220-7006 Email: us.info@cxbladder.com Web: www.cxbladder.com
CLIA #: 39D2053269 PA Permit #: 032894 MD Permit #: 1976 CA License #: COS 800426 FL License #: 800026956 RI License#: LCO00827 CAP 8714655

Cxbladder™ TRIAGE

PACIFIC EDGE DIAGNOSTICS USA LTD

Patient Name:
Patient Date of Birth:
Patient Hematuria History:
Patient Hematuria Status:
Patient Smoking History:
Patient Sex:
Medical Record #:
Sample ID:
Alternate ID:
Specimen Source:
Specimen Collection Date:
Specimen Receipt Date:
Specimen Report Date:
Report Status:
ICD-10 Code:
Ordering Clinician:

Test Result: Cxbladder Triage score

Comments:

Results Interpretation:

Cxbladder Triage Score <4.0	This test has a Negative Predictive Value (NPV) of 98.5%, and a Sensitivity of 95.1%. Low probability of urothelial carcinoma (UC).
Cxbladder Triage Score ≥4.0	Continue with the clinician-directed standard clinical workup to establish if urothelial carcinoma (UC) is present.

The Cxbladder Triage test was developed and internally validated on 587 patients from three clinical cohorts of patients presenting with macrohematuria, and evaluated on a cohort of 40 patients presenting with microhematuria. The proportion of patients without urothelial carcinoma (UC) was 87.7%. With the test negative rate of 40%, the observed Negative Predictive Value (NPV) was 98.5% and the Sensitivity was 95.1% for the segregation of patients with a low probability of having UC. All false negatives were low-grade Ta tumors (WHO98 classification).

Cxbladder Triage has been developed and validated for the segregation of patients who have a low probability of having UC and is intended to be used in conjunction with standard clinical workup for patients presenting with hematuria who may not require further evaluation for UC. Interpretation of other conditions and/or malignancies in patients have not been validated.

Assay Description:

Cxbladder Triage is a qualitative assay for urothelial carcinoma (UC) that combines the quantitative measure of gene expression from five mRNA biomarkers (CDK1, MDK, IGFBP5, HOXA13, and CXCR2) as measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR) from a small sample of the patient's urine and four patient characteristics: patient age, sex, smoking history and hematuria history. An algorithm is applied to these nine parameters to calculate a Cxbladder Triage Score ranging from 0 to 10 and using a cut-off of 4.0 identifies those patients with a low probability of having UC. Cxbladder Triage is a development from the clinical study published in BMC Urology (2015) 15:23.

Reviewed By: Thomas P. Nifong, MD (electronically signed)
Laboratory Director, Pacific Edge Diagnostics USA Ltd

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CLIA #: 39D2053269 PA Permit #: 032894 MD Permit #: 1976 CA License #: COS 800426 FL License #: 800026956 RI License#: LCO00827 CAP 8714655

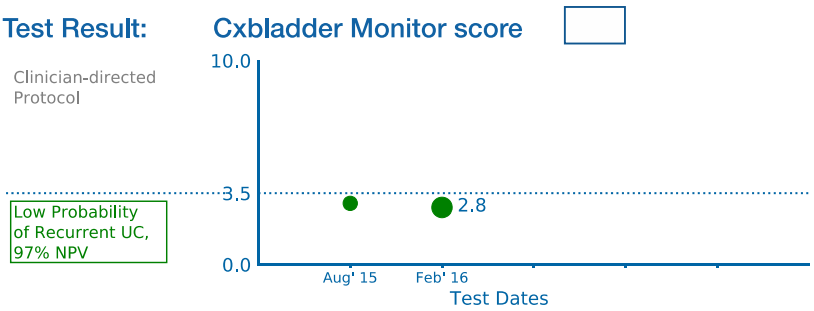
Cxbladder™ MONITOR

PACIFIC EDGE DIAGNOSTICS USA LTD

Patient Name:
Patient Date of Birth:
Patient Sex:
Medical Record #
Date of Last UC Diagnosis:
Last UC Diagnosis:

Specimen Source:
Sample ID:
Alternate ID:
Specimen Collection Date:
Specimen Receipt Date:
Specimen Report Date:
Report Status:
ICD-10 Code:
Ordering Clinician:

Test Result:



Comments:

Results Interpretation:

Cxbladder Monitor Score ≥ 3.5	A clinician-directed protocol to determine the presence of recurrent UC, is warranted.
Cxbladder Monitor Score < 3.5	This test has a Negative Predictive Value (NPV) of 97%, and a sensitivity of 93%. Low probability of recurrent UC.

The Cxbladder Monitor test was developed as a 'rule out' test for recurrence of urothelial carcinoma (UC). A total of 763 patients were recruited (1,036 samples) in a multicenter clinical study of patients undergoing routine clinical surveillance for recurrent UC. A classifier was created that uses two variables (time since last UC diagnosis, and whether the last event was primary or recurrent UC) combined with the concentrations of five mRNA biomarkers (CDK1, MDK, IGFBP5, HOXA13 and CXCR2) to identify those patients who have a low probability of recurrent UC. The classifier was validated using statistically robust iterative methods. A Cxbladder Monitor score less than 3.5 has a Negative Predictive Value (NPV) of 97% and a sensitivity of 93%, with a test negative rate of 34%. Cxbladder Monitor is a development from the clinical study published in J. Urology (2017) 197:1419-1426.

Assay Description:

Cxbladder Monitor is a quantitative assay for recurrent UC that combines the gene expression from five mRNA biomarkers (CDK1, MDK, IGFBP5, HOXA13 and CXCR2) as measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR) from a small sample of urine and two clinical factors associated with the patients prior history of UC. An algorithm is applied to these parameters to calculate a Cxbladder Monitor score ranging from 0 to 10, and using a cut-off of 3.5 identifies those patients who have a low probability of recurrent UC.

Reviewed By: Thomas P. Nifong, MD (electronically signed)
Laboratory Director, Pacific Edge Diagnostics USA Ltd

Disclaimer: This is a high complexity clinical test developed by and its performance characteristics determined by Pacific Edge Ltd. This test is for clinical purposes and is intended to aid the clinician to rule out the presence of recurrent urothelial carcinoma (UC). It should not be regarded as investigational or for research only. This test has not been approved by the US Food and Drug Administration (FDA) and such approval is not required. The laboratory is regulated under Clinical Laboratory Improvement Amendments of 1988 (CLIA).

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Cxbladder MONITOR

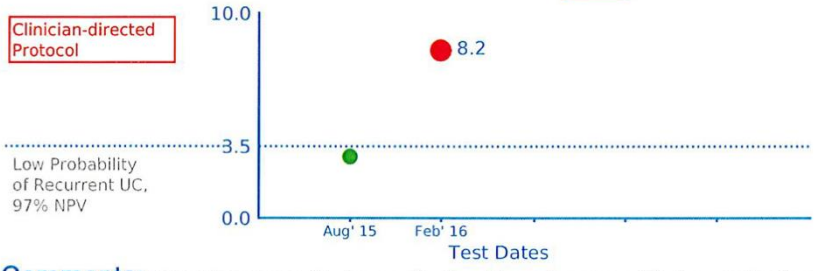
PACIFIC EDGE DIAGNOSTICS USA LTD

Patient Name:	Bridget von Hammersmark
Patient Date of Birth:	06/13/1964
Patient Sex:	Female
Medical Record #	232323
Date of Last UC Diagnosis:	May 2015
Last UC Diagnosis:	Recurrence
Specimen Source:	Urine
Sample ID:	PE000AHJ
Alternate ID:	722839
Specimen Collection Date:	02/01/2016
Specimen Receipt Date:	02/03/2016
Specimen Report Date:	02/04/2016
Report Status:	**Final**
ICD-10 Code:	R31.9
Ordering Clinician:	Dr Hugo Stiglitz

Dr Hugo Stiglitz
Louisiane Medical Clinic
1214 Research Blvd.
Suite 2000
Hummelstown, PA, 17036

Test Result: Cxbladder Monitor score 8.2

95% CI (7.80 - 8.80)



Comments: Example comment that spans the line. Example comment that spans the line. Example comment that spans the line.

Results Interpretation:

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Cxbladder Monitor Score < 3.5	This test has a Negative Predictive Value (NPV) of 97%, and a sensitivity of 93%. Low probability of recurrent UC.

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Assay Description:

Cxbladder Monitor is a quantitative assay for recurrent UC that combines the gene expression from five mRNA biomarkers (CDK1, MDK, IGFBP5, HOXA13 and CXCR2) as measured by reverse transcription quantitative polymerase chain reaction (RT-qPCR) from a small sample of urine and two clinical factors associated with the patients prior history of UC. An algorithm is applied to these parameters to calculate a Cxbladder Monitor score ranging from 0 to 10, and using a cut-off of 3.5 identifies those patients who have a low probability of recurrent UC.

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Laboratory Director, Pacific Edge Diagnostics USA Ltd

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Cxbladder™

Sample Collection Manual

Sample Collection

CRITICAL ELEMENTS FOR SAMPLE COLLECTION

- Check expiration date on USS box, do not use if expired
- The test requires a **FRESH** mid-stream urine sample, preferably the second void of the day.
- The sample must be taken prior to cystoscopy, bladder wash, catheterization, or any treatment and transferred to the Cxbladder tube within **ONE HOUR**.
- Please collect **only midstream** voided samples. Bladder wash/barbotage specimens are not acceptable as they may result in false positives.
- The sample should be collected in a **1st use collection cup.** Do not collect sample through any re-usable device such as a flow meter or re-usable cup.
- Prior to transferring any sample from a specimen cup to the tube, please **INVERT or AGITATE** the specimen to remove cells off of the bottom of the sample cup.
- Samples with **excessive blood or unusual discoloration** could be rejected.
- Cxbladder tube **must be filled** to the fill line on the tube or the sample will be rejected. Requires > 4.5 mls of urine
- Write **patient's full name and date of birth** on the sample collection tube
- **Do not add any preservative or chemical** to the urine

PATIENT CONSIDERATIONS

- **Inflammatory Conditions May Cause a No Result Cxbladder Report**
- Patients undergoing any manipulation, or invasive urothelial cancer detection should have a voided urine specimen collected first OR wait **6 weeks** from the date of the procedure prior to using Cxbladder.
- Patients undergoing any intrathecal therapy that causes an immune response (i.e. BCG/ chemotherapy) should wait **6 weeks** post last therapy prior to using Cxbladder.
- **UTI/Bladder Infection** – Patients with an active bladder infection of ***any kind*** could cause a no result Cxbladder report due to high inflammation or extremely high bacteria counts. Please wait **2 weeks** after the infection has cleared before using the Cxbladder test.

Urine Sample Collection

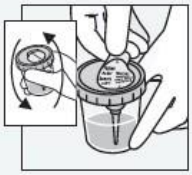
1

Check expiration date on tube, do not use if expired. Label the Cxbladder tube with patient's name, date of birth and the date of sample collection. **Must list this information in addition to barcode on the tube.**

2

Collect mid-stream urine into the Cxbladder urine collection cup until at least 1/3 full. When completed, screw the lid back on the cup securely. **Must be transferred to Cxbladder urine tube within 1 hour of sample collection.**

3



Prior to transferring the urine into the Cxbladder tube, **invert the Cxbladder urine collection cup several times** and then peel back the white protective sticker on the cup to expose the rubber covered needle.

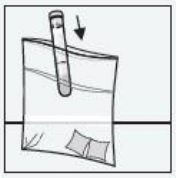
4

Place the Cxbladder urine collection cup on a flat, hard surface (like a desk). Push the Cxbladder tube onto the rubber-covered needle and hold in position until the flow stops **at fill line on tube.** Remove tube and **invert several times.** Do not open the tube as it contains a corrosive buffer.

5

Remove the lid from the Cxbladder urine collection cup and dispose of it in sharps collector. Dispose of urine and collection cup according to your facility's policy.

6



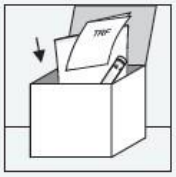
Place the Cxbladder tube into the plastic specimen bag containing absorbent pads and seal.

7

Paperwork

- Complete **all** areas of the TRF form as instructed in the TRF specifics section on the back of this card.
- Have **ordering clinician sign** Statement of Medical Necessity

8



Place specimen bag and **all** paperwork inside the Cxbladder box, place in FedEx shipping bag, and complete waybill.

DO NOT WRITE ANYTHING ON OUTSIDE OF BOX.

9

Ship sample within 2 days so that it arrives at laboratory within 7 days of collection. PEDUSA FEDEX NUMBER:154316620 / For FedEx Pickup call: 800-463-3339. When scheduling a pickup, press "0" until you reach a live representative.

02-MKT-023-US-REV-C

Cxbladder®

TEST REQUISITION FORM

Pacific Edge Diagnostics USA Ltd
1214 Research Boulevard, Suite 2000
Hummelstown, PA 17036 USA
Phone: 1-855-CXBLADR (1-855-292-5237)
Fax: 1-717-220-7006

BARCODE

Processing Code _____

I. SPECIMEN INFORMATION

Test ordered: ☑ Cxbladder Detect ☐ Cxbladder Monitor ☐ Cxbladder Triage

In Office Collection Date (MM/DD/YY) MM/DD/YY In-Home Target Sample Date(MM/DD/YY) _____

Automatic Replacement ☐ Send In-Home Collection System if first sample is Rejected or Qualifying No Result

ICD-10 Code: ☑ R31.0 Gross Hematuria ☐ R31.1 Benign Essential Microscopic Hematuria ☐ R32.21 Asymptomatic Microscopic Hematuria
☐ R31.29 Other Microscopic Hematuria ☐ C67.9 Malignant Neoplasm of Bladder ☐ Z85.51 Hx of Bladder Cancer
☐ Other _____

II. PATIENT INFORMATION

Last Name LAST NAME First Name FIRST NAME MI _____

DOB (MM/DD/YY) MM/DD/YY Sex ☑ Male ☐ Female Medical Record # _____

Address _____ City _____ State _____ Zip _____

Phone _____ ☑ Attached copy of patient's information

III. IMPORTANT PATIENT HISTORY (Required information to generate a Cxbladder Monitor Result)

☐ Yes ☐ No Does the patient have a prior history of Urothelial Carcinoma (UC)?

If No, then request Cxbladder Detect or Cxbladder Triage

What was the date of the patients most recent confirmed tumor? (MM/DD/YY) _____

What was the most recent tumor ? (Check one) ☐ Initial tumor (primary disease) ☐ Recurrent tumor (recurrent disease)

IV. IMPORTANT PATIENT HISTORY (Required information to generate a Cxbladder Triage Result)

Hematuria History (answer both questions)

☐ Yes ☐ No Does the patient have microscopic hematuria?

☐ Yes ☐ No Has the patient had gross hematuria (visible blood in the urine) more than once per day anytime within the last 12 months?

Smoking History

☐ Yes ☐ No Has the patient smoked more than 100 cigarettes in his/her lifetime?

V. BILLING INFORMATION

☐ OPTION 1: Bill Insurance
☑ Copy of the patient's insurance information is attached

Primary Insurance Name _____

Phone _____

Fax _____

Member ID # _____

Group No # _____

Authorization # _____

Secondary Insurance: Does patient have a secondary insurance?

☐ NO ☐ YES, attach a copy of patient's insurance card (front and back)

☐ OPTION 2: Self Pay

Patient agrees to pay for the test and will be billed directly by Pacific Edge Diagnostics USA LTD at the address indicated above.

VI. ORDERING CLINICIAN INFORMATION

Last Name LAST NAME

First Name FIRST NAME

Practice PRACTICE NAME

NPI # NPI NUMBER

Phone PHONE-NUMBER

Address PRACTICE ADDRESS

City CITY State STATE Zip ZIP

Fax FAX-NUMBER

Email _____

The Cxbladder Test Report will be sent by mail and fax unless one of the following is checked:

☐ Mail Only ☑ Fax Only ☐ Secure Email Only

VII. AUTHORIZATION

I confirm the Cxbladder test is medically necessary for the diagnosis or detection of a disease, illness, impairment, symptom, syndrome or disorder, and the results will be used in the medical management and treatment decisions for the patient. I have obtained the patient's consent for Pacific Edge Diagnostics USA Ltd to release the Cxbladder test results to the patient's third party payer when necessary as part of the reimbursement process and the patient's consent for authorization of payment of medical benefits to Pacific Edge. I confirm that the person listed in the ORDERING CLINICIAN INFORMATION (section VI) is authorized by law to order the test(s) requested herein.

☑ **I authorize my printed name above to serve as a digital signature**

Clinician Signature PRINT CLINICAN'S NAME Date (MM/DD/YY) MM/DD/YY

SN 1-4-0009

INSTRUCTIONS FOR COMPLETING THIS FORM AND SHIPPING SPECIMEN ARE INCLUDED ON THE BACK 07-QMS-059-REV-A

Clear Form

Statement of Medical Necessity

Physician Name:	First and Last Name		
Patient Name:	First and Last Name	**Date of Birth:**	MM/DD/YY

Test Name (check one)

- (●) **Cxbladder® Detect (CPT 0012M)** *A urine-based test incorporating the levels of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2) into an algorithm to calculate a risk score for urothelial carcinoma.*
- ( ) **Cxbladder® Monitor (CPT 0013M)** *A urine-based test incorporating the levels of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2) into an algorithm, along with risk factors of time since diagnosis and previous recurrence, to calculate a risk score for recurrent urothelial carcinoma.*
- ( ) **Cxbladder® Triage (CPT 0363U)** *A urine-based test incorporating the levels of five genes (MDK, HOXA13, CDC2 [CDK1], IGFBP5, and CXCR2) into an algorithm, along with risk factors of smoking history and macrohematuria frequency, to calculate a risk score for urothelial carcinoma.*

I ordered Cxbladder® to aid in the evaluation of this patient for urothelial carcinoma because during my examination I have determined that the patient has an increased risk of having cancer due to:

- (●) The presence of Gross Hematuria
- ( ) The presence of Microscopic Hematuria
- ( ) An abnormal or atypical urine cytology result
- ( ) An inconclusive cystoscopic evaluation
- ( ) A history of Urothelial Carcinoma undergoing Surveillance
- ( ) An inconclusive result from testing performed with other diagnostic tests (FISH, etc)
- ( ) Other:_____

I intend to use the results of Cxbladder® along with other outcomes from my urologic evaluation:

- (●) To determine if cystoscopy and/or medical imaging are indicated to further evaluate this patient for urothelial carcinoma
- ( ) To determine if cystoscopy can be avoided in this patient with a history of cystoscopic complications
- ( ) To determine if the interval between surveillance cystoscopies can be increased or a cystoscopy can be skipped during patient's surveillance.
- ( ) To determine whether invasive procedures such as biopsies are necessary to further evaluate the patient for urothelial carcinoma
- ( ) Other:_____

Informed Consent and Statement of Medical Necessity - I confirm that the patient listed above has been informed that Cxbladder® is being requested as part of their evaluation. I consider Cxbladder® to be reasonable and medically necessary for the evaluation of this patient, and intend to use the results in the medical management or treatment decisions. I confirm that I am both the ordering and treating provider.

Provider Signature: First and Last Name Date: MM/DD/YY

Form MUST be returned to Pacific Edge Diagnostics USA, Ltd. via fax: 1-717-220-7006 and included in the patient's medical record.

References:

1. Kavalieris L, O'Sullivan P, Frampton C, Guilford P, Darling D, Jacobson E, et al. Performance Characteristics of a Multigene Urine Biomarker Test for Monitoring for Recurrent Urothelial Carcinoma in a Multicenter Study. J Urol. 2017;197(6):1419-26. Epub 20161214. doi: 10.1016/j.juro.2016.12.010. PubMed PMID: 27986532.
2. O'Sullivan P, Sharples K, Dalphin M, Davidson P, Gilling P, Cambridge L, et al. A multigene urine test for the detection and stratification of bladder cancer in patients presenting with hematuria. J Urol. 2012;188(3):741-7. Epub 20120719. doi: 10.1016/j.juro.2012.05.003. PubMed PMID: 22818138.
3. Lotan Y, O'Sullivan P, Raman JD, Shariat SF, Kavalieris L, Frampton C, et al. Clinical comparison of noninvasive urine tests for ruling out recurrent urothelial carcinoma. Urol Oncol. 2017;35(8):531.e15-.e22. Epub 20170331. doi: 10.1016/j.urolonc.2017.03.008. PubMed PMID: 28366272.
4. Kavalieris L, O'Sullivan PJ, Suttie JM, Pownall BK, Gilling PJ, Chemasle C, et al. A segregation index combining phenotypic (clinical characteristics) and genotypic (gene expression) biomarkers from a urine sample to triage out patients presenting with hematuria who have a low probability of urothelial carcinoma. BMC Urol. 2015;15:23. Epub 20150327. doi: 10.1186/s12894-015-0018-5. PubMed PMID: 25888331; PubMed Central PMCID: PMC4391477.
5. Lotan Y, Raman JD, Konety B, Daneshmand S, Schroeck F, Shariat SF, et al. Urinary Analysis of *FGFR3* and *TERT* Gene Mutations Enhances Performance of Cxbladder Tests and Improves Patient Risk Stratification. J Urol. 2023;209(4):762-72. Epub 20221230. doi: 10.1097/JU.0000000000003126. PubMed PMID: 36583640.
6. Woldu SL, Ng CK, Loo RK, Slezak JM, Jacobsen SJ, Tan WS, et al. Evaluation of the New American Urological Association Guidelines Risk Classification for Hematuria. J Urol. 2021;205(5):1387-93. Epub 20201224. doi: 10.1097/ju.0000000000001550. PubMed PMID: 33356483.
7. Teoh JY, Kamat AM, Black PC, Grivas P, Shariat SF, Babjuk M. Recurrence mechanisms of non-muscle-invasive bladder cancer - a clinical perspective. Nat Rev Urol. 2022;19(5):280-94. Epub 20220331. doi: 10.1038/s41585-022-00578-1. PubMed PMID: 35361927.
8. Li KD, Chu CE, Patel M, Meng MV, Morgan TM, Porten SP. Cxbladder Monitor testing to reduce cystoscopy frequency in patients with bladder cancer. Urol Oncol. 2023;41(7):326.e1-.e8. Epub 20230302. doi: 10.1016/j.urolonc.2023.01.009. PubMed PMID: 36868882.
9. Holyoake A, O'Sullivan P, Pollock R, Best T, Watanabe J, Kajita Y, et al. Development of a multiplex RNA urine test for the detection and stratification of transitional cell carcinoma of the bladder. Clin Cancer Res. 2008;14(3):742-9. doi: 10.1158/1078-0432.ccr-07-1672. PubMed PMID: 18245534.
10. Proposed LCD - Genetic Testing for Oncology (DL39365). https://www.cms.gov/medicare-coverage-database/view/lcd.aspx?lcdId=39667&ver=92023.
11. Rai BP, Luis Dominguez Escrig J, Vale L, Kuusk T, Capoun O, Soukup V, et al. Systematic Review of the Incidence of and Risk Factors for Urothelial Cancers and Renal Cell Carcinoma Among Patients with Haematuria. Eur Urol. 2022;82(2):182-92. Epub 20220405. doi: 10.1016/j.eururo.2022.03.027. PubMed PMID: 35393159.

12. Edward M. Schaeffer RHL, Sandy Srinivas, Nabil, Adra YA, Daniel Barocas, Rhonda Bitting, Alan Bryce, Brian Chapin, Heather, H. Cheng AVDA, Neil Desai, Tanya Dorff, James A. Eastham, Thomas, A. Farrington Xin Gao SG, Thomas Guzzo, Joseph E. Ippolito, Michael, R. Kuettel JML, Tamara Lotan, Rana R. McKay, Todd Morgan, George, Netto JMP-S, Robert Reiter,, et al. NCCN Guidelines Version 4.2023 Prostate Cancer. 2023.
13. Neeraj Agarwal SA, Hilary Bagshaw, Michael, Baine KB, Marian, Birkeland NB, Maria Carlo, Toni Choueiri, Brian Costello, Ithaar, Derweesh AD, Tanya Fischer, Yasser, Ged SG, John Gore, Kristina Gregory, Andrew Gunn, Naomi Haas, Evelyn, Handel EH, Anne Holbrook, Michael Johnson, Eric Jonasch, Payal Kapur,, et al. NCCN Guidelines Version 1.2024 Kidney Cancer. 2023.
14. Davidson PJ, McGeoch G, Shand B. Inclusion of a molecular marker of bladder cancer in a clinical pathway for investigation of haematuria may reduce the need for cystoscopy. N Z Med J. 2019;132(1497):55-64. Epub 20190621. PubMed PMID: 31220066.
15. Davidson PJ, McGeoch G, Shand B. Assessment of a clinical pathway for investigation of haematuria that reduces the need for cystoscopy. N Z Med J. 2020;133(1527):71-82. Epub 20201218. PubMed PMID: 33332329.
16. Barocas DA, Boorjian SA, Alvarez RD, Downs TM, Gross CP, Hamilton BD, et al. Microhematuria: AUA/SUFU Guideline. J Urol. 2020;204(4):778-86. Epub 20200723. doi: 10.1097/ju.0000000000001297. PubMed PMID: 32698717.