

# MEDCO FORUM<sup>®</sup>

PRESENTING INNOVATIVE PRODUCTS AND SERVICES TO HEALTHCARE PROFESSIONALS

VOLUME 15 NUMBER 25

MAY 2008

REPRINT

## TRENDS IN FISH TESTING FOR BLADDER CANCER DETECTION

by Kirk J. Wojno, MD

**U**roVysion<sup>™</sup> fluorescence in situ hybridization (FISH) molecular testing for bladder cancer has gained acceptance in the diagnosis and follow-up of bladder cancer patients. Although originally proposed as a binary test with positive and negative results, it is a much more powerful tool of risk stratification as a continuous variable. The centromeric fluorescent probes detect increased chromosomal number for chromosomes 3—red, 7—green, and 17—aqua. Homozygous loss of the locus-specific probe 9p21, which harbors a tumor suppressor gene, is also indicative of urothelial neoplasia. Both multisomy of chromosomes 3, 7, 17 or homozygous loss of 9p21 at the appropriate levels are independently associated with urothelial neoplasms and carcinoma.

The urine is a very complicated environment, with many cell types to evaluate, and significant differences between the sexes. For example, female urine has significantly more squamous cells than typically observed in the male. Determining the appropriate cell type for enumeration under the fluorescent dark field environment can be challenging. Inflammation can be associated with increased numbers of umbrella cells and urothelial tissue fragments, each with their own inherent interpretative issues. Reactive phenomenon are associated with increased entry into the cell cycle, as well as binucleation. These can lead to increased numbers of cells with tetrasomy for all four probes including 9p21 which is typically lost in neoplasia. Caution must be

*... the correlation  
between UroVysion,  
cytology, and histology  
is essential to proper  
treatment planning,  
especially in patients  
with low level  
positive results.*

exercised when evaluating cases just into the positive range based on the presence of a few tetraploid cells.

Tetraploid FISH signal patterns may be associated with both malignant and non-malignant conditions. Only a relatively small percent (2-10%) of bladder cancers are tetraploid. The non-malignant conditions may include: G2 phase of normal cell division resulting from a reactive process such as inflammation; drugs (fluoroquinolones); or radiation therapy. Post-instrumentation specimens may also lead to preferential sampling of the deeper layers of the urothelium, which are more apt to be in the cell cycle. Therefore, the diagnostic value of tetraploid FISH signal patterns may be limited in predicting true positive recurrent cases. The significance of tetraploid

FISH signal patterns in hematuria patients is uncertain. Results with tetraploid cells should be interpreted with caution.

Automated microscopy has eliminated some of the subjectivity in cellular screening approaches, and has facilitated the examination of larger nuclei first. Overlapping nuclei, split signals, and stringency-related splatter remain challenges for the automated systems, necessitating visual review of digital images. In general, the automated systems have increased interpretative reproducibility for this test. Subjectivity in determining appropriate cell populations for enumeration has recently been aided by the use of immunolabeling for cytokeratin 7 in the research setting. This staining has increased the efficiency of finding abnormal cells.


Test offerings have evolved into three patterns: the straight UroVysion; urine cytology with concurrent UroVysion; and UroVysion that is reflexively done for cases with abnormal cytology. A recent study of reflex UroVysion testing showed utility in cases that had abnormal cytology. There was no added benefit in cases that were obviously positive by either cystoscopy and or cytology. The reflexively-run UroVysion for abnormal cytology maximizes specificity and helps to avoid unnecessary aggressive workups, especially for denovo microhematuria patients. UroVysion

with concurrent cytology maximized sensitivity and specificity most appropriate for following patients with treated bladder cancer. This is especially useful since the inflammation associated with BCG therapy does not seem to interfere with the test. UroVysion as a stand-alone test maximized sensitivity for high-grade disease, and is most useful for persistent hematuria or symptomatology in high-risk patients or patients with a history of bladder cancer.

One of the functional limitations of the UroVysion test is its ultrasensitivity; it may detect urothelial neoplasia at a very early stage before it can be detected by traditional techniques. These may represent the so-called anticipatory positive or small lesions that can be handled by the body's immune surveillance system. UroVysion is also limited in its ability to detect low-grade, low-stage lesions. However, these non-aggressive lesions are readily detected by cystoscopy.

Although the test is typically reported as negative or positive, additional numeric detail is usually given in the report. Additional key considerations—above finding sufficient cells for a positive result (typically four)—are the denominator and percentage of positive cells. This information allows for risk stratification and appropriate management decisions. Following the specific probe, numerical aberrations may allow for

differentiation of multicentricity from multiclonality.

In summary, the correlation between UroVysion, cytology, and histology is essential to proper treatment planning, especially in patients with low-level positive results. In general, the test is most useful in allowing for prolonged screening intervals in patients being followed for bladder cancer who have a negative test. 

### To Learn More

To learn more about UroVysion™ at AmeriPath, please visit the Web site at [www.ameripath.com](http://www.ameripath.com), or call 1-800-330-6565.

### References:

1. Pajor G, Sule N, Alpar D, Kajtar B, Kneif M, Bollmann D, Somogyi L, Pajor L. Increased efficiency of detecting genetically aberrant cells by UroVysion test on voided urine specimens using automated immunophenotypical preselection of uroepithelial cells. *Cytometry A*. 2008 Mar;73(3):259-65.
2. Steiner H, Bergmeister M, Verdorfer I, Granig T, Mikuz G, Bartsch G, Stoehr B, Brunner A. Early results of bladder-cancer screening in a high-risk population of heavy smokers. *BJU Int*. 2008 Mar 11.
3. Hajdinjak T. UroVysion FISH test for detecting urothelial cancers: Meta-analysis of diagnostic accuracy and comparison with urinary cytology testing. *Urol Oncol*. 2008 Jan 14.
4. Lotan Y, Bensalah K, Ruddell T, Shariat SF, Sgalowsky AI, Ashfaq R. Prospective Evaluation of the Clinical Usefulness of Reflex Fluorescence In Situ Hybridization Assay in Patients With Atypical Cytology for the Detection of Urothelial Carcinoma of the Bladder. *J Urol*. 2008 Apr 16.
5. Gofrit ON, Zorn KC, Silvestre J, Shalhav AL, Zagaja GP, Msezane LP, Steinberg GD. The predictive value of multi-targeted fluorescent in-situ hybridization in patients with history of bladder cancer. *Urol Oncol*. 2008 May-Jun;26(3):246-9. Epub 2008 Feb 20.
6. Daniely M, Rona R, Kaplan T, Olsfanger S, Elboim L, Freiburger A, Lew S, Leibovitch I, BioView Ltd., Rehovot, Israel. Grossman HB. Combined morphologic and fluorescence in situ hybridization analysis of voided urine samples for the detection and follow-up of bladder cancer in patients with benign urine cytology. *Urol Oncol*. 2008 May-Jun;26(3):332.