

## **FISH for the Detection of Bladder Cancer in Urine Specimens**

### Disclosure

*Dr. Halling receives grant funding from Abbott Molecular, Inc., to develop FISH assays for the detection of neoplastic cells in cytologic specimens. He holds a patent on the UroVysion™ probe set and receives royalties.*

### Introduction

The Papanicolaou stain has been the primary modality utilized to identify tumor cells in cytologic specimens for over half a century. However, ancillary techniques such as immunocytology, DNA ploidy analysis by flow cytometry/image cytometry, and fluorescence in situ hybridization (FISH) have been increasingly utilized to identify neoplastic cells in cytologic specimens in recent years.

Tumors result from genetic and epigenetic alterations that activate oncogenes and inactivate tumor suppressor genes. These mutations occur at both the chromosomal and molecular level. Examples of chromosomal alterations that occur in tumors include aneuploidy, deletion, amplification, and translocation.

### Fluorescence In Situ Hybridization

FISH utilizes fluorescently-labeled DNA probes to detect various types of chromosomal alterations in cells including aneusomy (i.e. abnormalities of chromosome copy number), duplications, amplification, deletion, and translocation. FISH probes can be broadly categorized as chromosome enumeration probes (CEP) or locus specific indicator (LSI) probes. CEP probes are used to detect aneusomy whereas LSI probes are generally used to detect deletion, duplication, or amplification of specific genes. FISH is able to detect cells that have chromosomal abnormalities consistent with neoplasia in exfoliative and aspiration cytology specimens, and FISH is now widely used to detect bladder cancer in urine specimens and will likely soon be used to identify tumor cells in a variety of other cytologic specimens.

### UroVysion™ (Sensitivity and Specificity; Clinical Indications)

UroVysion™ (Abbott Molecular, Inc., Des Plaines, IL) is a FISH probe set that was developed for the detection of bladder cancer (1). This probe set contains CEP probes for chromosomes 3, 7, and 17, and an LSI probe to the 9p21 band that are labeled with red, green, aqua, and gold fluorophores, respectively. A recent review of the published literature comparing the performance of FISH with UroVysion to conventional cytology reveals that UroVysion is more sensitive than cytology for all grades and stages of urothelial carcinoma (2). The lower sensitivity of UroVysion for low-grade tumors is primarily due to the fact low-grade tumors are usually diploid or near-diploid tumors with relatively few chromosomal abnormalities(3, 4). However, failing to detect some low-grade tumors is less of a concern than missing high-grade tumors since low-grade tumors behave less aggressively.

The specificity of FISH with UroVysion observed in these same studies was slightly lower than the specificity (85% vs. 93%) of urine cytology (2). However, the UroVysion assay is quite sensitive, and it is not uncommon for the assay to be positive for a patient in

whom tumor cannot be identified. Such cases could either represent false positive UroVysion results or a failure of cystoscopy to identify a lesion that is present. Several studies have shown that UroVysion can detect recurrent UC before it is clinically evident by cystoscopy (5, 6).

UroVysion received FDA approval for monitoring bladder cancer patients for tumor recurrence in 2001 and for assessing patients with hematuria (gross and microscopic) for bladder cancer in 2005(5, 7). However, UroVysion should not be used indiscriminately for the evaluation of any patient with hematuria. For example, it is the opinion of this author that it should not be used for patients under 45 years of age with microhematuria but no other risk factors for bladder cancer. The reason that UroVysion should not be used for such patients is that even a test with relatively good specificity can have a low positive predictive value when used in very low disease prevalence population. It is recommended that when using UroVysion to evaluate patients with hematuria, that it mainly be used on patients who have other risk factors for bladder cancer such as a current or past smoking history and age greater than 45. When used in such patients, the positive predictive value of a positive result will be quite high.

#### UroVysion™ (Performing and Interpreting)

FISH with UroVysion has been performed using various types of urine specimens including voided urine, urine obtained by catheterization, bladder and ureteral washings, and stomal urine specimens. However, the FDA approved specimen is voided urine. A detailed explanation of how to perform the UroVysion FISH assay is provided with the UroVysion test packet insert. Non-neoplastic cells generally show two copies for each of the four probes with the UroVysion probe set. However, a small fraction (<10% usually) of normal cells may show only one copy of one or more of the four probes due to signal overlap or incomplete hybridization. Urothelial carcinoma cells, on the other hand, will show one of several types of chromosomal abnormality which include polysomy, tetrasomy/near-tetrasomy, trisomy, and 9p21 loss(2).

Polysomic cells are cells that show gains (i.e. more than two copies) for two or more of the four probes. Tetrasomic cells are a specific type of polysomic cell which have four copies of all four probes. Trisomic cells exhibit three copies of one probe (most commonly CEP 7) and two or fewer copies of the other three probes. Cells with homozygous 9p21 loss alone show no copies of the 9p21 probe and two copies of the other three probes. Receiver operator curves have been used to determine the optimal cutoff for considering a case positive when using polysomy as the criterion for positivity. These curves have revealed that the finding of four or more cells with polysomy was associated with a sensitivity of 85% and a specificity of 95%(1).

Although tumors can be tetraploid and shed tetrasomic cells into the urine, we are more conservative in our interpretation of cases as showing tetrasomy. This is because non-neoplastic cells in the S, G2, and M phases of the cell's cycle can show tetrasomic or near tetrasomic signal patterns. For this reason, our threshold for positivity for tetrasomy is 10 or more cells showing tetrasomy. This cutoff appears to be associated with relatively good specificity (i.e. relatively few false positive results).

The criterion for positivity for homozygous 9p21 loss that are widely used were established during the original UroVysion FDA trial (5). This trial demonstrated that the finding of 12 or more of the 25 most morphologically abnormal cells with homozygous 9p21

loss was associated with acceptable sensitivity and specificity. In our practice, we are very conservative about calling cases positive for 9p21 loss for two major reasons: 1) the 9p21 probe is the weakest of the four probes and there is a tendency for inexperienced technologists to over interpret 9p21 loss, 2) “overcalling” 9p21 loss will lead to unnecessary clinical evaluation of a patient who even if he had true 9p21 loss, would likely have at most a relatively benign low-grade tumor.

In our practice, ~95% of the cases which are considered positive for abnormality exhibit polysomy. Homozygous 9p21 deletion and tetrasomy make up most of the remaining 5% of positive cases. Trisomy is uncommon and constitutes <1% of the positive cases. Polysomy generally correlates with the presence of a higher grade tumor, whereas, homozygous 9p21 loss alone generally correlates with the presence of a low-grade papillary tumor. Little data is available regarding the clinical correlates of tetrasomy and trisomy diagnoses.

#### Potential Use for Patients Being Treated with BCG

BCG, a live weakened strain of *Mycobacterium bovis*, is frequently used to treat superficial bladder cancer. Unfortunately, BCG induces an intense inflammatory reaction that makes the interpretation of cystoscopic findings difficult since areas of erythema can represent either carcinoma in situ or inflammation. Cytologic interpretation is also complicated by reactive changes in the cells that make it difficult to distinguish malignant from reactive non-neoplastic cells(8). A recent study suggests that UroVysion may be useful for assessing response to treatment in superficial bladder cancer patients receiving BCG or other intravesical therapy (9). This study found that BCG did not interfere with the interpretation of UroVysion results and that UroVysion was able to identify patients that had a higher risk of tumor recurrence and muscle invasive disease. Patients with a positive FISH result at the end of their treatment were 4.6 times more likely to develop recurrent tumor and 9.4 times more likely to develop muscle-invasive tumor than patients with a negative FISH result.

There is only one published study that has assessed the utility of UroVysion for the detection of upper tract UC (10), and UroVysion assay does not, at the current time, have FDA approval for this indication. Consequently, if a laboratory wishes to use it for this purpose, they should perform their own internal validation before clinically implementing. In our experience, an abundance of tetrasomic cells is a fairly common finding in upper tract washing specimens, even in patients who do not appear to have tumor. For this reason, we are especially conservative about interpreting the finding of tetrasomic cells in upper tract specimens as evidence of tumor except when such cells are present in high percentages. In contrast, we have found that the finding of four or more cells with hyper-tetrasomic signal patterns (i.e. cells in which at least one of the four probes shows five or more copies) has high specificity for the presence of UC and interpret such findings as consistent with a diagnosis of UC or other tumor involving the upper tract.

#### Impact of BK Infection (“Decoy Cells”) on Test Results

Renal transplant patients often develop a recrudescence of latent BK polyomavirus infections and shed polyomavirus infected cells (“decoy cells”) into the urine. Interestingly, studies have shown that decoy cells, which can mimic UC cells, are markedly aneuploid when assessed for ploidy status by digital image analysis. This curious finding made us wonder if such cells might cause false positive FISH results. To evaluate this, we assessed

38 patients with decoy cells in their urine for DNA ploidy status using DIA and for chromosomal abnormalities with UroVysion and found that 84% of the cases showed aneuploidy by DIA but only 13% showed chromosomal abnormalities by FISH (11). None of the patients were known to have UC. The patients with positive FISH results had the highest viral titers. Thus, it appears that polyomavirus infection may occasionally cause false positive FISH results, but this occurs primarily in patients with very high BK viral titers.

### Conclusions

FISH using UroVysion represents a promising new way of detecting bladder cancer in urine specimens. Most studies have shown that FISH with UroVysion has significantly higher sensitivity than conventional cytology for the detection of bladder cancer. Nonetheless, FISH is more time consuming and expensive to perform than conventional cytology. Consequently, it is important to utilize FISH testing primarily for patients that are most likely to benefit. FISH analysis of cytologic specimens, utilizes morphologic skills, and cytologists are ideally suited for the analysis and interpretation of these specimens. However, if cytopathology laboratories are going to perform this testing, it will be important for cytologists to become increasingly knowledgeable about cancer genetics and its application to diagnostic cytology.

### Take Home Points

- ✓ UroVysion has FDA approval for monitoring bladder cancer patients for tumor recurrence and for assessing patients with hematuria for bladder cancer
- ✓ UroVysion is more sensitive but slightly less specific than conventional urine cytology.
- ✓ Cytologists are ideally suited for the analysis and interpretation of UroVysion but need to become increasingly knowledgeable about cancer genetics and its application to diagnostic cytology.

### **References**

1. Sokolova, I. A., Halling, K. C., Jenkins, R. B., Burkhardt, H. M., Meyer, R. G., Seelig, S. A., and King, W. The development of a multitarget, multicolor fluorescence in situ hybridization assay for the detection of urothelial carcinoma in urine. *Journal of Molecular Diagnostics*, 2: 116-123, 2000.
2. Halling, K. C. and Kipp, B. R. Fluorescence in situ hybridization in diagnostic cytology. *Human Pathology*, 38: 1137-1144, 2007.
3. Bittard, H., Lamy, B., and Billery, C. Clinical evaluation of cell deoxyribonucleic acid content measured by flow cytometry in bladder cancer. *Journal of Urology*, 155: 1887-1891, 1996.
4. Richter, J., Jiang, F., Gorog, J. P., Sartorius, G., Egenter, C., Gasser, T. C., Moch, H., Mihatsch, M. J., and Sauter, G. Marked genetic differences between stage pTa and stage pT1 papillary bladder cancer detected by comparative genomic hybridization. *Cancer Research*, 57: 2860-2864, 1997.

5. Sarosdy, M. F., Schellhammer, P., Bokinsky, G., Kahn, P., Chao, R., Yore, L., Zadra, J., Burzon, D., Osher, G., Bridge, J. A., Anderson, S., Johansson, S. L., Lieber, M., Soloway, M., and Flom, K. Clinical evaluation of a multi-target fluorescent in situ hybridization assay for detection of bladder cancer. *Journal of Urology*, *168*: 1950-1954, 2002.
6. Yoder, B. J., Skacel, M., Hedgepeth, R., Babineau, D., Ulchaker, J. C., Liou, L. S., Brainard, J. A., Biscotti, C. V., Jones, J. S., and Tubbs, R. R. Reflex UroVysion testing of bladder cancer surveillance patients with equivocal or negative urine cytology: a prospective study with focus on the natural history of anticipatory positive findings. *American Journal of Clinical Pathology*, *127*: 295-301, 2007.
7. Sarosdy, M. F., Kahn, P. R., Ziffer, M. D., Love, W. R., Barkin, J., Abara, E. O., Jansz, K., Bridge, J. A., Johansson, S. L., Persons, D. L., and Gibson, J. S. Use of a multitarget fluorescence in situ hybridization assay to diagnose bladder cancer in patients with hematuria. *Journal of Urology*, *176*: 44-47, 2006.
8. Mack, D. and Frick, J. Diagnostic problems of urine cytology on initial follow-up after intravesical immunotherapy with Calmette-Guerin bacillus for superficial bladder cancer. *Urol Int*, *52*: 204-207, 1994.
9. Kipp, B. R., Karnes, R. J., Brankley, S. M., Harwood, A. R., Pankratz, V. S., Sebo, T. J., Blute, M. M., Lieber, M. M., Zincke, H., and Halling, K. C. Monitoring intravesical therapy for superficial bladder cancer using fluorescence in situ hybridization. *Journal of Urology*, *173*: 401-404, 2005.
10. Akkad, T., Brunner, A., Pallwein, L., Gozzi, C., Bartsch, G., Mikuz, G., Steiner, H., and Verdorfer, I. Fluorescence in situ hybridization for detecting upper urinary tract tumors--a preliminary report. *Urology*, *70*: 753-757, 2007.
11. Kipp, B. R., Sebo, T. J., Griffin, M. D., Ihrke, J. M., and Halling, K. C. Analysis of polyomavirus-infected renal transplant recipients' urine specimens: correlation of routine urine cytology, fluorescence in situ hybridization, and digital image analysis. *American Journal of Clinical Pathology*, *124*: 854-861, 2005.