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| Patient Name: John Doe | Requesting Physician: David A. Smith, M.D. | Collected: 03/13/2009 |
| Sex: Male | Client/Group Facility: Urology Associates | Received: 03/20/2009 |
| Date of Birth (Age): 10/10/1958 (50 years) | Additional Recipient: Mark Howard, M.D. | Reported: 03/23/2009 |
| SSN: 212-22-2234 | Submitting Pathologist: Tim Jackson, M.D. | Case #: C09-123445 |
| Patient ID: 23445921 | Specimen ID: C09-22311 | Req #: |

UroVysion™ Assay with Semi-Quantitative Analysis

CLINICAL HISTORY

History of bladder cancer. Completed BCG 02/01/2009.

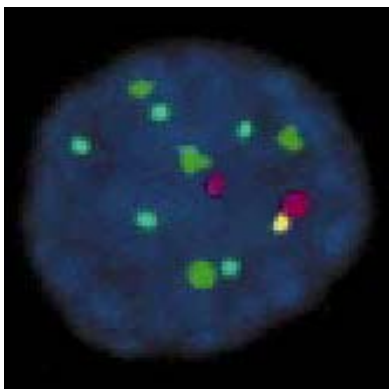
RESULTS

POSITIVE. Enumeration of DNA targets revealed greater than or equal to 4 non-tetrasomic cells showing gains for two or more chromosomes 3, 7, and 17 in the same cell.

INTERPRETATION

High-Risk FISH Results. Enumeration of 100 consecutive, non-inflammatory cells revealed a non-tetrasomic polysomy of more than one chromosome 3, 7, and 17 in 35 (35%) of cells. These findings are most consistent with urothelial carcinoma in situ, invasive urothelial carcinoma, or high-grade papillary non-muscle invasive carcinoma with increased risk of tumor recurrence and progression to muscle invasive cancer.¹⁻² Less likely diagnoses include prostate carcinoma in males or metastatic cancer involving the genitourinary tract. Clinical and histological correlation is suggested.

IMAGE FROM BIOVIEW SYSTEM



Abnormal result observed in an aneusomic interphase cell obtained from a sample showing two copies of CEP 3 (red), four copies of CEP 7 (green), five copies of CEP 17 (aqua) and one copy of LSI 9p21 (gold) after the UroVysion™ multi-probe hybridization.

REFERENCE RANGE

POSITIVE

- 1) ≥4 non-tetrasomic cells showing gains for 2 or more chromosomes 3, 7, and 17 in the same cell;³ or
- 2) ≥10 cells showing tetrasomy/near-tetrasomy for chromosomes 3, 7, and 17;³ or
- 3) ≥10 cells showing gains for a single chromosome 3, 7 or 17;¹ or
- 4) ≥12 cells with homozygous loss of the 9p21 locus

UNINFORMATIVE

Insufficient specimen quality, inadequate slide preparation, or fewer than 25 evaluable cells.

LOW-RISK FISH

- 1) Negative-FISH result; or
- 2) Positive-FISH result
 - a. <5% cells demonstrating non-tetrasomic gains for 2 or more chromosomes 3, 7, and 17 in the same cell;² or
 - b. <10% cells showing tetrasomy/near-tetrasomy for chromosomes 3, 7, and 17;¹ or
 - c. <10% cells showing gains for a single chromosome 3, 7 or 17;¹
 - d. 9p21 loss¹

HIGH-RISK FISH

- 1) Positive-FISH result
 - a. ≥5% cells demonstrating non-tetrasomic gains for 2 or more chromosomes 3, 7, and 17 in the same cell (bladder cancer recurrence);² or
 - b. >10% cells demonstrating non-tetrasomic gains for 2 or more chromosomes 3, 7, and 17 in the same cell (progression to muscle-invasive cancer);² or
 - c. ≥10% cells showing tetrasomy/near-tetrasomy for chromosomes 3, 7, and 17 (bladder cancer recurrence and progression);¹ or
 - d. ≥10% cells showing gains for a single chromosome 3, 7 or 17 (bladder cancer recurrence and progression)¹

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ENUMERATION SUMMARY

| Chromosome 3, 7, 17 and 9p21 Locus | | Total number of cells analyzed: 100 | |
|--|-----------------|-------------------------------------|-----------------|
| Gains of multiple chromosomes (3, 7, and 17): | 40 (40%) | 9p21 loss: | 10 (10%) |
| Non-tetrasomy: | 35 (35%) | Homozygous: | 5 (5%) |
| Tetrasomy/Near-tetrasomy: | 5 (5%) | Heterozygous: | 5 (5%) |
| With 9p21 loss: | 5 (5%) | | |
| Gains of a single chromosome (3, 7 or 17): | 7 (7%) | | |
| Trisomy 3, 7 or 17 | 0 (0%) | | |
| Non-trisomy: | 7 (7%) | | |

Criteria for categorization of chromosome 3, 7, 17 copy number and 9p21 locus.

- 1) **Gains of multiple chromosomes:** cells that show gains (≥ 3 copies) for two or more of the three centromeric (CEP 3, CEP 7, and CEP 17) probes.
 - a. **Non-tetrasomy:** cells that show gains (3 or ≥ 5 copies) for two or more of the three centromeric (CEP 3, CEP 7, and CEP 17) probes.
 - b. **Tetrasomy:** cells that show four copies for each centromeric (CEP 3, CEP 7, and CEP 17) probes.
 - c. **Near-tetrasomy:** cells that show four copies for any two centromeric (CEP 3, CEP 7, and CEP 17) probes but three copies for one.
 - d. **Tetrasomy/Near-tetrasomy with 9p21 loss:** tetrasomic/near tetrasomic cells that show an absolute (i.e., homozygous/heterozygous) or relative loss of 9p21 signals.
- 2) **Gains of a single chromosome:** cells that show an isolated gain for one of the three centromeric (CEP 3, CEP 7 or CEP 17) probes.
 - a. **Trisomy:** cells that show three copies for one of the three centromeric (CEP 3, CEP 7 or CEP 17) probes but two or fewer copies of the other two probes.
 - b. **Non-trisomy:** cells that show gains (≥ 4 copies) for one of the three centromeric (CEP 3, CEP 7 or CEP 17) probes.
- 3) **9p21 loss:** cells can show a homozygous or heterozygous loss of 9p21 signals.
 - a. **Homozygous loss (9p21):** absence of both 9p21 signals.
 - b. **Heterozygous loss (9p21):** presence of only one 9p21 signal.

INTENDED USE

The UroVysion™ Bladder Cancer Kit (UroVysion™ Kit) is designed to detect aneuploidy for chromosomes 3, 7, 17, and loss of the 9p21 locus via fluorescence in situ hybridization (FISH) in urine specimens from persons with hematuria suspected of having bladder cancer. Results from the UroVysion™ Kit are intended for use, in conjunction with and not in lieu of current standard diagnostic procedures, as an aid for initial diagnosis of bladder carcinoma in patients with hematuria and subsequent monitoring for tumor recurrence in patients previously diagnosed with bladder cancer.

METHODOLOGY

Slide Preparation and Pretreatment. Urine specimens for UroVysion™ analysis are prepared using the liquid-based cytology technique (ThinPrep®, Cytoc, Boxborough, MA). The prepared specimen slides are protease digested, fixed in formaldehyde, washed, and dehydrated.

FISH Assay. The FISH assay is performed using a four-target, multicolor interphase FISH probe set. The FISH probe mixture consists of three alpha-satellite repeat sequence probes; chromosome enumeration probe (CEP) 3 SpectrumRed, CEP 7 SpectrumGreen and CEP 17 SpectrumAqua that hybridize to the centromere regions of chromosomes 3, 7, and 17, respectively. In addition, a unique sequence probe, locus-specific indicator (LSI) p16 SpectrumGold, is included that hybridizes to the 9p21 region of chromosome 9.

Enumeration of FISH Signals. Determination of results is conducted by enumeration of CEP 3, 7, 17 and LSI 9p21 through automated interphase FISH analysis using the Duet™ digital image analysis system (Bioview Ltd., Nes Ziona, Israel) equipped with appropriate excitation and emission filters allowing visualization of the red, green, aqua and gold fluorescent signals. Specimens hybridized with the UroVysion™ multi-color probe mixture will exhibit signals indicative of the copy number of chromosomes 3, 7, 17 and of the 9p21 region.

Interpretation of Results. A minimum of 25 non-inflammatory cells are analyzed. The signal distribution for cells showing either single or multiple gains of the chromosome 3, 7, 17 or loss of the 9p21 locus is recorded. The total number of chromosomally abnormal cells, i.e., cells with gains of chromosome 3, 7, 17 or loss of the 9p21 locus is determined and the results are reported as positive or negative along with semi-quantitative findings including pattern of chromosomal abnormality (i.e., gains of single/multiple chromosome(s), tetrasomy/near-tetrasomy, tetrasomy with 9p21 loss, trisomy, and homozygous/heterozygous 9p21 loss) and percent abnormal cells.

CLINICAL CONSIDERATIONS

Quantitative Molecular Cytology in the Surveillance of Non-muscle Invasive Urothelial Carcinoma. Numerous studies have shown that FISH, using the UroVysion™ probe set, has comparable specificity to and better sensitivity than routine cytology in the detection of bladder cancer in urine specimens.⁴⁻⁸ The UroVysion™ assay, as currently approved by the Food and Drug Administration (FDA), is simply reported

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qualitatively as positive or negative for abnormality. However, mounting data suggests that assessing FISH quantitatively may help to stratify the risk of recurrence and progression of non-muscle invasive bladder cancer.^{4,9-10}

A recent study by Kruger et al. showed numerical changes of chromosome 17 and the 9p21 locus to be independent predictors of stage Ta tumor recurrence.¹¹ A separate prospective study of intermediate-risk patients (those with T1G2, TaG2, or multifocal TaG1 lesions) found that 60% of those with aneuploidy of chromosome 7 and/or 17 developed recurrence. However, only 15% of patients with numerical alterations of chromosome 3 and/or the 9p21 locus had recurrence, indicating that some alterations may be more significant than others.¹⁰

In a retrospective cohort study, Kipp et al. demonstrated that among patients with a history of non-muscle invasive bladder cancer there is a positive correlation between the percentage of polysomic cells found in the urine by FISH and bladder cancer recurrence and progression to muscle invasive disease.² The percent abnormal by FISH was one of the most significant variables (HR 1.026, $P < 0.001$) for predicting recurrent bladder cancer with a 2.6% increased chance of having cancer for every 1% increase in the percentage of abnormal cells by FISH. Similarly, the percentage of abnormal cells was one of the most significant ($P < 0.001$) variables for identifying disease with a 1.8% increased risk of having muscle invasive cancer for every 1% increase in the percentage of abnormal cells by FISH.

Anticipatory Positive Results. The FISH assay is quite sensitive, and it is not uncommon for the assay to be positive for a patient in whom tumor cannot be identified. Several studies have now demonstrated that FISH can detect recurrent urothelial carcinoma before it is clinically evident by cystoscopy.^{4,5,7,9,12} Sarosdy et al. reported that there were 36 patients with a negative cystoscopic examination but a positive FISH result.⁴ With continued longitudinal follow-up, 15 (42%) of these cases were found to have biopsy-proven tumor recurrence, with time to tumor diagnosis of 3 to 16 months (mean, 6.0 months). Conversely, among 68 patients who had a negative cystoscopy and a negative FISH result, only 13 (19%) had a biopsy-proven recurrence at 3 to 19 months (mean, 11.2 months). The patients with a positive FISH result but negative cystoscopy were referred to as "anticipatory positive" cases. A Kaplan-Meier curve showed that the time to tumor recurrence was significantly less for patients with anticipatory positive FISH results compared with those with negative FISH results. A recent report by Yoder et al.⁵ found that approximately 27% of patients with a negative or atypical cytology result had a positive FISH result but no evidence of tumor. However, approximately 65% of these patients were found to have tumor recurrence within 29 months, which further suggests that positive FISH results cannot be ignored despite an absence of clinically detectable tumor.

Monitoring Intravesical Therapy. Surveillance cystoscopy and cytology after Bacillus-Calmette Guerin (BCG) therapy can be difficult to interpret due to inflammatory changes in the urothelium. Kipp et al. used FISH to assess the response to therapy in patients with superficial bladder cancer receiving BCG or other intravesical therapies.¹³ This study demonstrated that BCG did not interfere with the interpretation of FISH results and that FISH 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 result.

Risk Stratification and Surveillance Strategies. Ongoing experience with the UroVysion™ FISH assay suggest that the pattern of chromosomal aneuploidy and percent abnormal cells in a positive FISH assay itself may have clinical significance.^{1,2} The results obtained with the FISH may be used not only to detect FISH-associated evidence of urothelial cancer but also to distinguish between patients with low-risk and high-risk non-muscle invasive bladder cancer. Based on the chromosomal pattern and percentage of abnormal cells, low-risk patients with an anticipated uneventful clinical course and/or recurrent low-grade papillary tumors can be distinguished from those high-risk patients who are more prone to tumor progression. More aggressive assessments and a shortened interval between examinations may be advisable for patients with high-risk FISH results, whereas patients with low-risk FISH results could undergo longer intervals between invasive cystoscopic monitoring.

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If you have any questions on this report please do not hesitate to contact the BioVantra client support center at (866) 627-8221.

Peter A. Tsivis, M.D.

Pathologist Electronic Signature

03/23/2009

Date

This test was developed and its performance characteristics determined by BioVantra, LLC. It has not been cleared or approved by the U.S. Food and Drug Administration (FDA). However, the FDA has determined that such approval is not necessary. This test is permitted for clinical purposes and should not be regarded as purely investigational or for research. BioVantra is certified under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) as qualified to perform high-complexity clinical testing.

Photomicrograph is a symbolic representation of the key findings of the UroVysion™ Assay report. The photomicrograph is not intended to replace a complete review and reading of the final diagnostic report provided.