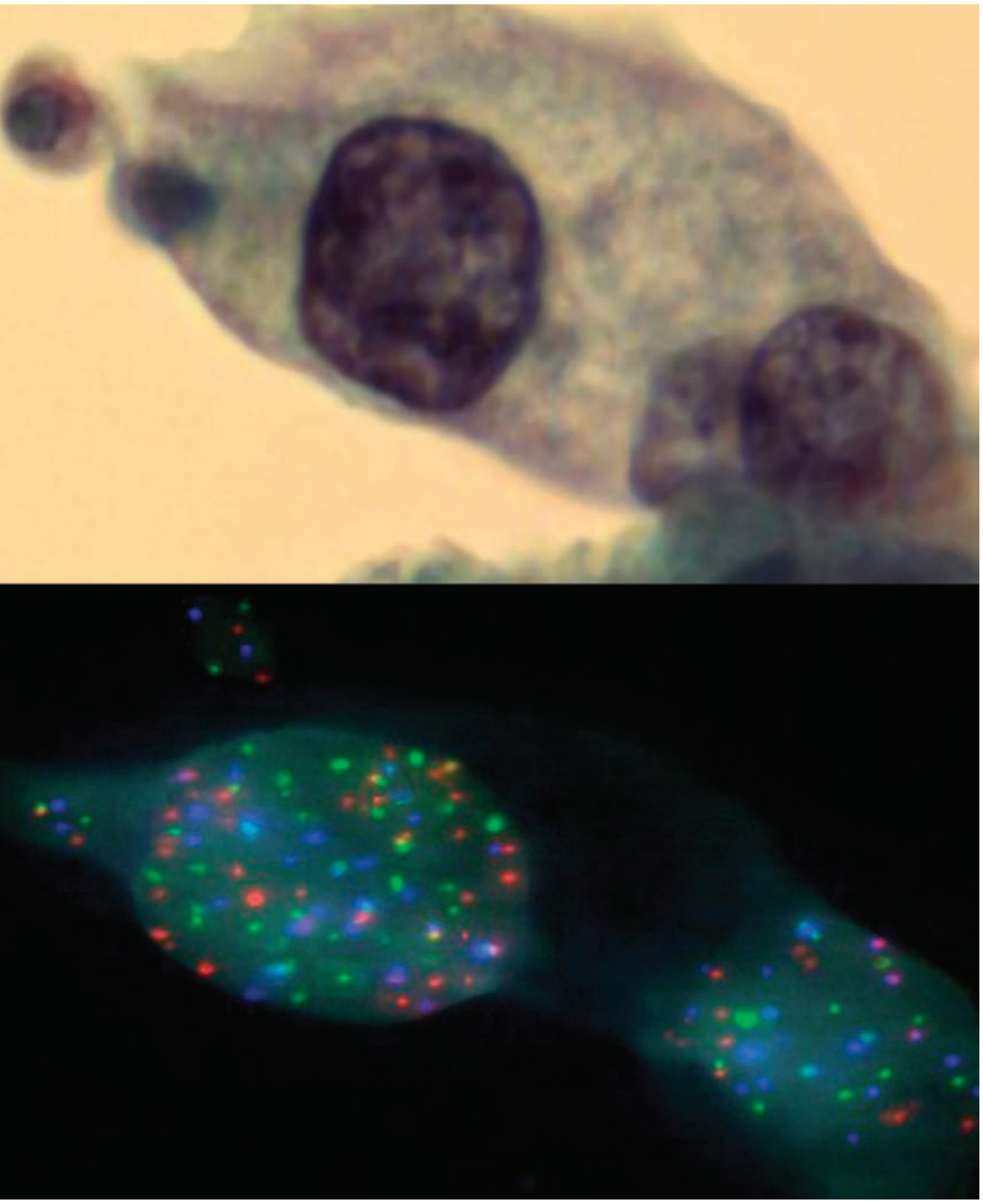
Issue 243-10

Anatomic Pathology Report

UMass Memorial Medical Center Anatomic Pathology Laboratories



Inside this issue:

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Photos (cover, left)

Top Panel – Atypical Urine Cytology: Papanicolaou (PAP) stained cell with large nucleus and increased nucleus/cytoplasm ratio.

Bottom Panel – UroVysion™ FISH of cells above: Abnormal pattern showing multiple copies of chromosomes 3 (red), 7 (green) and 17 (aqua).

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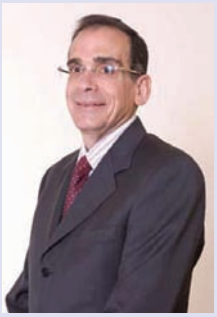

Message from the Director:

Welcome to the Inaugural edition of our Anatomic Pathology Reports. These reports will present information on timely topics in Diagnostic Pathology. I hope that you enjoy them and I welcome any feedback.

In this issue we review the adoption of an HPV genotyping algorithm adopted by the American Society for Colposcopy and Cervical Pathology. In a second article we will discuss how our laboratory has modified the Urovysion FISH assay to increase its sensitivity and specificity.

Bruce Woda, MD

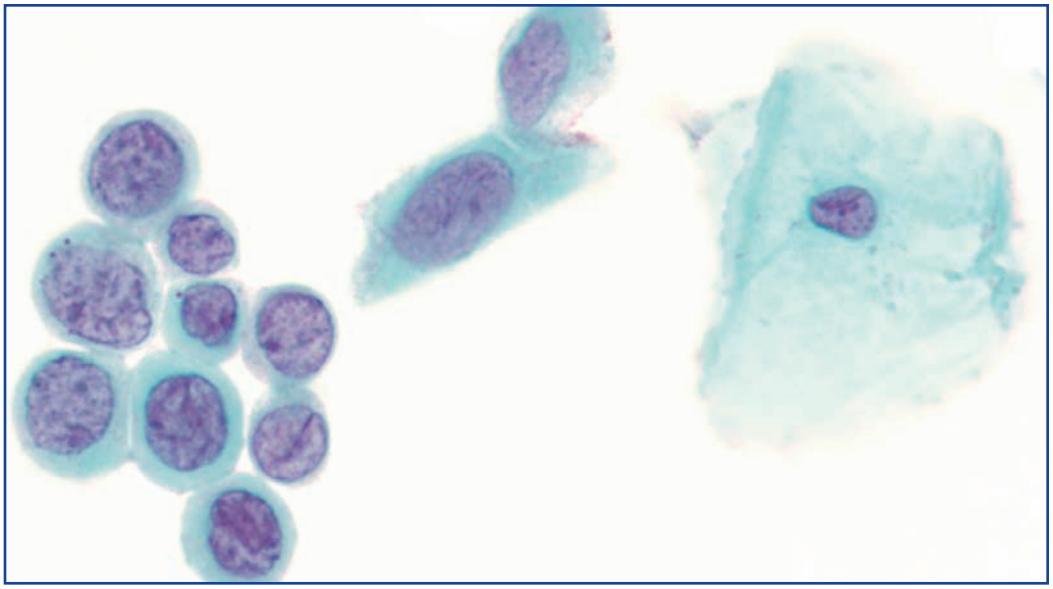

Photo (top, right)
GYN Papanicolaou (PAP) cytology: Group of small cells on the left and two cell in center are High Grade Squamous Intraepithelial Lesion (HGSIL). On the right, there is one normal squamous epithelial cell.

New Algorithm for Human Papilloma Virus (HPV) Testing

For several years, molecular testing for high-risk (HR) oncogenic types of HPV has been approved by the FDA and recommended by the American Society for Colposcopy and Cervical Pathology (ASCCP) for use as an adjunct to cervical cytology for screening in women 30 years of age and older. Sensitivity using a combination of HPV testing and cytology is significantly higher than that of either test alone with negative predictive values of 99-100%.[1, 2] Women who are negative by both cytology and HPV testing have a less than 1 in 1000 risk of having CIN 2 or greater,[3, 4, 5] and ordinarily do not need to be rescreened before 3 years,[6] http://www.asccp.org/consensus.shtml).

The HPV 16/18 genotypes are estimated to cause 70% of cervical cancers[7]. In cytology-negative women, the cumulative risk of a high grade squamous intraepithelial lesions (CIN3+), is ~20% when HPV 16/18 is positive versus 1.5% when HPV 16/18 is negative.[5] In recognition of this risk, the ASCCP released 2006 Consensus Guidelines for HPV genotyping, contingent on a FDA-approved genotyping assay becoming available,[6] Earlier this year the FDA approved the use of Cervista™ HPV 16/18 Invader® assay, a new HPV DNA diagnostic test specifically designed to detect HPV 16 and HPV 18.

The Laboratory of Diagnostic Molecular Oncology now offers a new algorithm for HPV testing. Any requests for the "ThinPrep® Pap test and HPV testing" will be initially screened for 13 high risk types of HPV (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68). Confirmatory testing for HPV 16/18 genotypes will be offered for HPV HR positive/Cytology negative specimens from women 30 years and older. ASCCP guidelines specify that cytology negative women 30 years and older who are HPV 16/18 positive should be referred for immediate colposcopy. Cytology negative women 30 years and older who are HPV HR positive but HPV 16/18 negative can be followed-up with repeat cytology and high-risk HPV testing in 12 months.[6] See Figure 1 on page 3.

Use of HPV Genotyping to Manage HPV HR *Positive/Cytology Negative Women 30 Years and Older

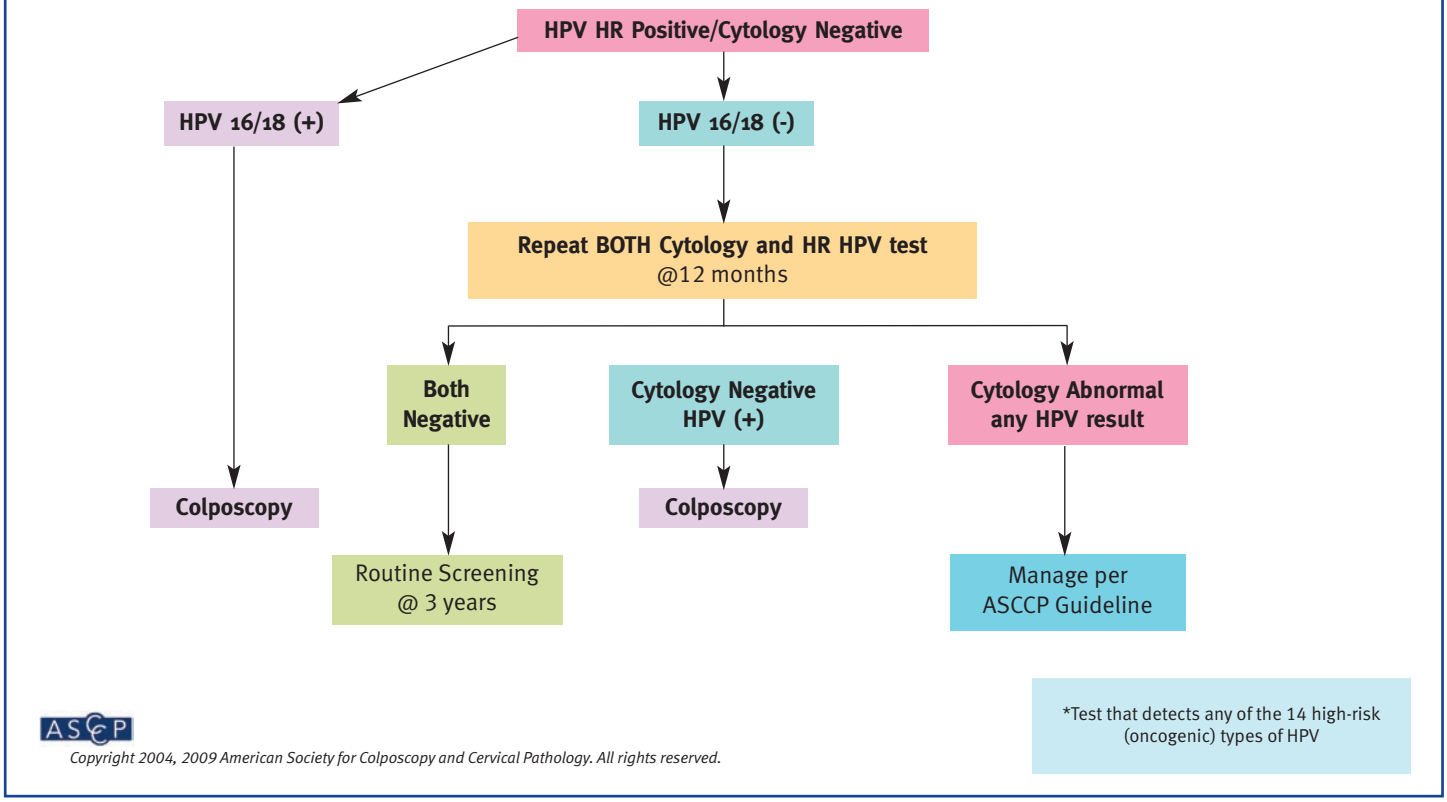

Figure 1: Algorithm for using HPV genotyping for HPV 16 and 18 to triage high-risk HPV positive/cytology negative women

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1. Cuzick J et al. Overview of the European and North American studies on HPV testing in primary cervical cancer screening. *Int J Cancer* 2006;119:1095-101.
2. *ACOG practice bulletin.* Clinical Management Guidelines for Obstetrician-Gynecologists. Number 61, April 2005. Human papillomavirus. *Obstet Gynecol* 2005;105:905-18.
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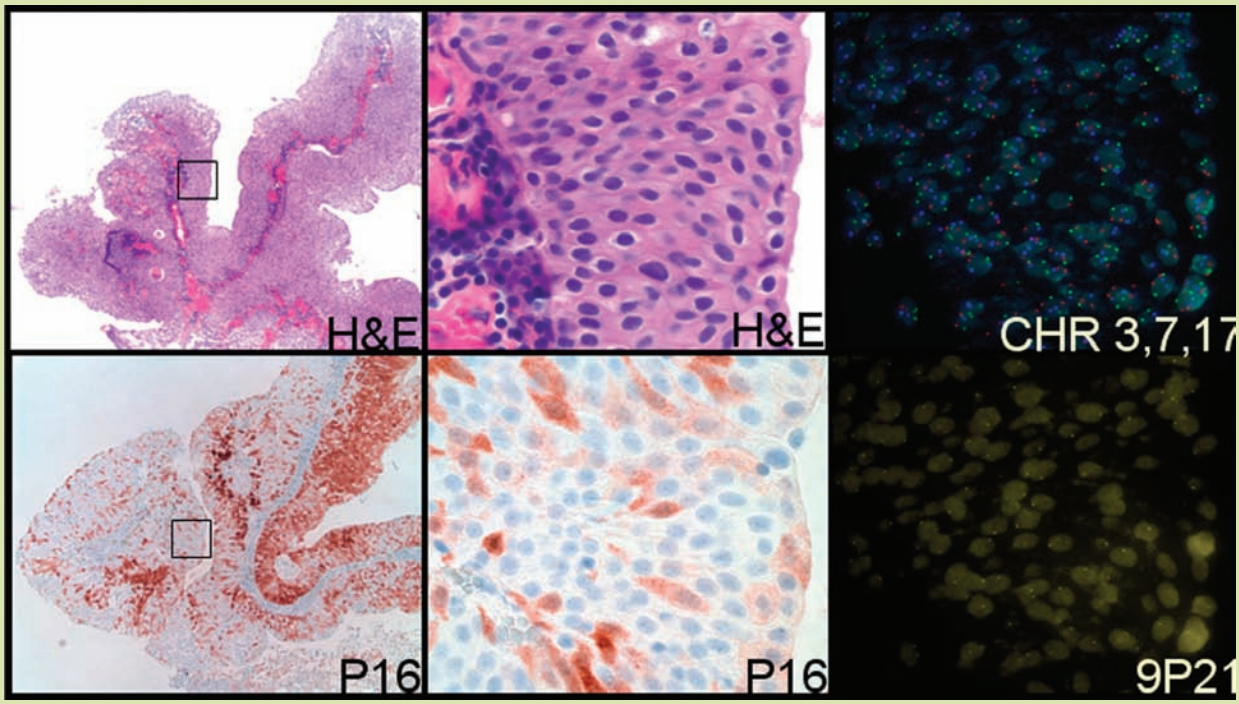
7. Munoz N et al. Epidemiologic Classification of Human Papillomavirus Types Associated with Cervical Cancer. *N Engl J Med* 2003; (Feb 6) 348:518.

If you have questions, comments or suggestions, please contact:

Ediz Cosar, M.D., *Medical Director, Diagnostic Molecular Oncology,* at 508-793-6240

Lloyd Hutchinson, Ph.D. *Technical Director, Diagnostic Molecular Oncology,* at 508-793-6240

Andrew Fischer, M.D., *Medical Director, Cytology Laboratory,* at 508-793-6140



Bladder Cancer Tissue FISH:

This low grade papillary bladder tumor sections show a heterozygous loss of the 9p21 locus (single copy of the P16/CDKN2A gene). This gene deletion has resulted in reduced expression of the p16 tumor suppressor protein. Sequential tissue sections were used for tissue morphology (H&E, x100, x1000 magnification), P16 immunohistochemistry (P16, x100, x1000 magnification), and UroVysionTM FISH (9p21/p16 = gold, chromosome 3 = red, 7 = green, 17 = aqua, x1000 magnification).

Urovysion Update

Bladder cancer is among the most common 5 cancers with approximately 70,000 new cases and nearly 14,000 deaths reported annually[1] in the United States. Approximately 50% of cases recur within 2 years of the diagnosis with an overall recurrence rate of 70%, making this cancer one of the most expensive cancers to treat[2].

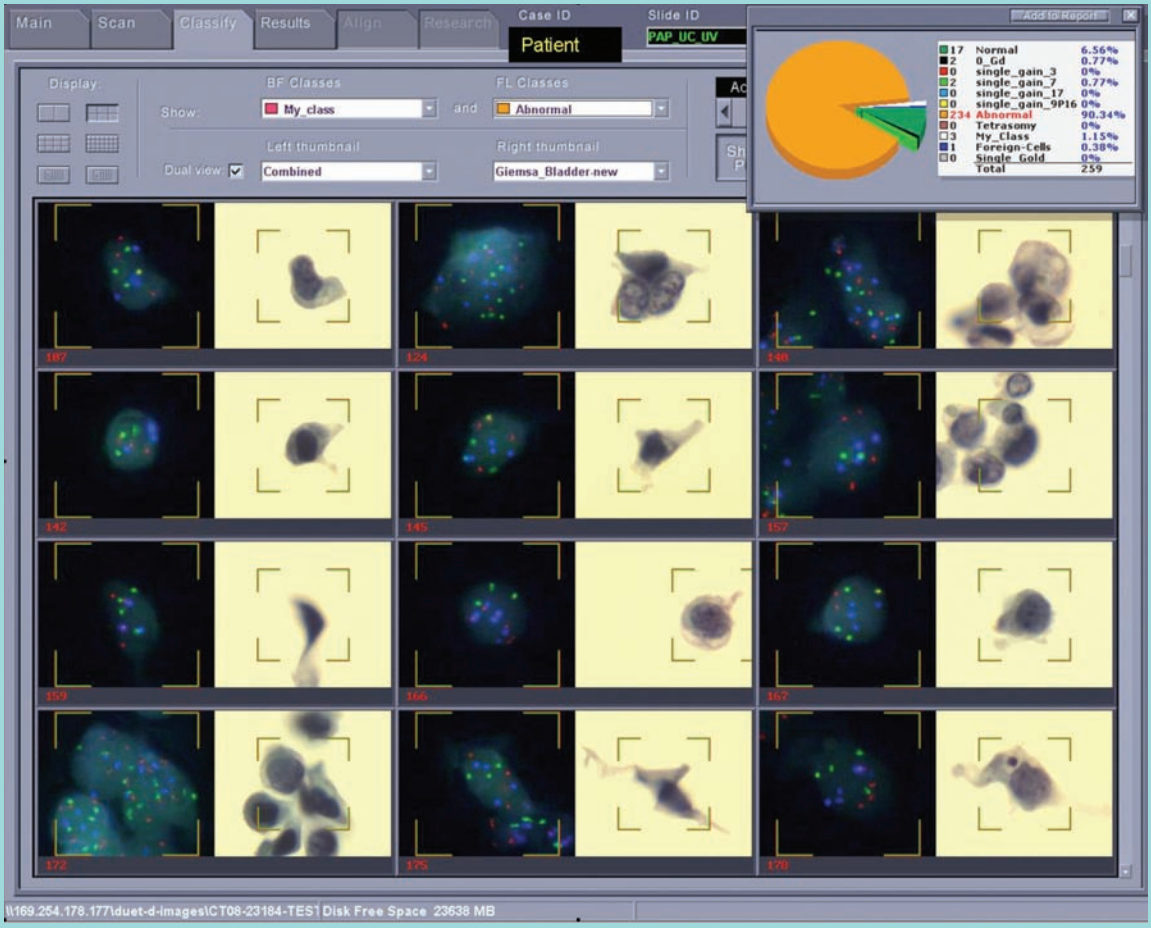
Various traditional clinical and laboratory modalities have shown limited value in the early detection of this potentially deadly disease. The most commonly used screening method for bladder cancer is urine cytology, which involves microscopic examination of the urine for malignant cells. Although this screening method is practical and has a high specificity (approximately 90%), it suffers from low sensitivity (approximately 30%), particularly in the detection of low grade bladder cancers. Due to subtle cytomorphologic features of particularly low grade urothelial carcinomas, cytologic examination not infrequently results in inconclusive diagnosis of “atypical urothelial cells”, resulting in unnecessary cystoscopic examination of the urinary bladder and other parts of the urinary tract. Over the past two decades several new promising markers have become available, all with the idea to overcome the inherent diagnostic limitation of the cytologic examination. These include antigen-based methods (BTA-stat, NMP22, ImmunoCyt/uCyt, CXCL1, MMP), molecular methods (telomerase, microsatellite analysis) and DNA ploidy analysis by flow cytometry. However, these methods have shown limited success in improving the sensitivity. Recently, fluorescent in situ hybridization (FISH), a molecular cytogenetic technique, has been introduced as a promising tool for the diagnosis of urothelial carcinoma.

Most malignant tumors including bladder cancers have altered genetic features, and frequently display copy number changes of chromosomes. Alterations in chromosomes can be detected by certain molecular techniques, such as FISH.

In 2005, the Food and Drug Administration (FDA) approved the application of FISH (UroVysion™ Bladder Cancer Kit by Abbott Molecular Laboratories, Downers Grove, IL) to urine samples to aid in the diagnosis of bladder cancer in patients who present with hematuria and in monitoring patients who have been diagnosed with bladder cancers. FISH utilizing a dark field microscope assesses copy number changes of chromosomes 3, 7, 17 and p16 gene locus in the short arm of chromosome 9 (9p21), most commonly affected chromosomes in bladder and other urinary tract cancers. A "positive" FISH result for the diagnosis of urothelial carcinoma is defined by the following criteria:

1. Four (4) or more urothelial cells with numerical gains in two (2) or more chromosomes.
2. Homozygous deletion of chromosome 9p21 in twelve (12) or more urothelial cells.

In 2006, the Department of Pathology at UMass Memorial Medical Center started to offer the FISH assay in the diagnosis of bladder and other urinary tract cancers. The system (Duet™ by BioView Ltd, Rehovot, Israel) that we use is more advantageous than the traditional dark field fluorescent microscope used for FISH, as it allows us to integrate FISH and bright field microscopic examination of urine cytology on the same urine sample (see figure below).



UroVysion™ FISH Classification:

An automated microscope scanning system (BioView™) enables combined analysis integrating cytology and FISH. Nuclei are sorted into different groups (e.g. normal nuclei show two copies of each probe). Sophisticated software allows the pathologist to review the case confirming that the urothelial cells have been analyzed and classified correctly. The urothelial cells in this example yielded an abnormal result (chromosome polysomy with 3 to 4 copies of each probe). The follow-up biopsy showed high-grade urothelial carcinoma.

ThinPrep™ Slide Preparation:
Cells present in the voided urine specimen are processed onto a ThinPrep™ slide and stained with Papanicolaou (PAP). The ThinPrep™ slide is reviewed by Pathologist, atypical cells identified and reflexes the specimen for FISH testing.

Duet Microscope:
The automated Duet microscope scanning system (BioView™) photographs cells in brightfield mode and sorts the cells into different classifications for review (e.g. atypical cells). Subsequently slides are hybridized for FISH and re-analyzed on the Duet system. Nuclei are automatically photographed and classified based on the UroVysion™ probe signal pattern (e.g. Abnormal).

Ongoing studies performed in the Laboratory of Diagnostic Molecular Oncology in the Department of Pathology at UMass Memorial Medical Center have shown improved sensitivity and specificity compared to conventional cytology or FISH alone. An integrated analysis provided a sensitivity of approximately 60% for low grade and approximately 95% for high grade urothelial carcinomas, and a specificity of approximately 95%.

In addition, studies have shown that UroVysion™ FISH is valuable in the early detection of recurrence of bladder cancer in biopsy negative cases[3,4,5] and in predicting response to BCG therapy[6].

Urine FISH utilizing UroVysion™ Bladder Cancer Detection Kit can be requested by selecting one of the options listed in Cytopathology or Diagnostic Molecular Oncology requisition forms (see below):

- **Urine Cytology only**
- **Urine Cytology and FISH, if cytology atypical**
- **Urine Cytology and FISH**
- **Urine FISH**

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6. Kipp B. J. *Urol,* 2005; 173: 401-404.

More information can be obtained from the Laboratory of Diagnostic Molecular Oncology (see contact information below) in the Department of Pathology, Division of Anatomic Pathology at UMass Memorial Medical Center.

Laboratory of Diagnostic Molecular Oncology
Department of Pathology | Division of Anatomic Pathology
UMass Memorial Medical Center
One Innovation Drive, Three Biotech
Worcester, MA 01605
Phone: (508) 793-6240

Anatomic Pathology Faculty

Bruce A. Woda, MD
Professor of Pathology and Vice Chair
Department of Pathology
Chief, Anatomic Pathology
Director of Hematopathology
Specialty: Hematopathology: Flow Cytometry, Molecular Diagnostics

Ali Akalin, MD, PhD
Assistant Professor of Pathology
Director, Head and Neck Pathology
Specialty: Head and Neck Pathology Pulmonary Pathology

Gamze Ayata, MD
Assistant Professor of Pathology
Director, GI Pathology
Specialty: Cytopathology, Surgical Pathology/ GI & Liver Pathology

Ediz F. Cosar, MD
Assistant Professor of Pathology
Medical Director, Diagnostic
Molecular Oncology
Specialty: Molecular Diagnostics, Cytology, Surgical Pathology

Yogeshwar Dayal, MD
Clinical Professor of Pathology
Specialty: GI Pathology

April Deng, MD, PhD
Associate Professor of Pathology
Director, Dermatopathology
Specialty: Dermatopathology

Andrew H. Fischer, MD
Professor of Pathology
Director, Cytopathology
Director, Cytopathology Fellowship Program
Specialty: Cytopathology

Armando E. Fraire, MD
Professor of Pathology
Director, Pulmonary Pathology and
Autopsy Pathology
Specialty: Pulmonary Pathology

Rujing Han, MD
Assistant Professor of Pathology
Specialty: Dermatopathology

Suyang Hao, MD
Clinical Associate Professor of Pathology
Director, Flow Cytometry
Director, Hematopathology
Fellowship Program
Specialty: Hematopathology and Flow Cytometry

Lloyd Hutchinson, PhD
Instructor in Pathology
Technical Director,
Diagnostic Molecular Oncology

Zhong Jiang, MD
Professor of Pathology
Director, Pathology Residency Program
Director, GU Pathology
Specialty: Genitourinary Pathology

Dina H. Kandil, MD
Assistant Professor of Pathology
Specialty: Breast, Cytology, Surgical Pathology

Ashraf Khan, MD
Professor of Pathology
Director, Surgical Pathology;
Breast and Endocrine Pathology
Director, Surgical Pathology
Fellowship Program
Specialty: Breast and Endocrine Pathology

Elizabeth Kurian, MD
Assistant Professor of Pathology
Specialty: Cytopathology

Di Lu, MD
Associate Professor of Pathology
Director, GYN Pathology and
Immunohistochemistry
Specialty: Anatomic Pathology, GYN Pathology

Nader A. Morad, MD, PhD
Clinical Associate Professor of Pathology
Specialty: Surgical Pathology

Christopher Owens, MD
Assistant Professor
Specialty: GU, Cytology, Surgical Pathology

Lan Peng, MD
Assistant Professor of Pathology
Specialty: Surgical Pathology ,GI and Liver Pathology

Jerzy Sobota, MD
Instructor of Pathology
Laboratory Medical Director
Wing Memorial Hospital

Thomas W. Smith, MD
Professor of Pathology
Director, Neuropathology and
Electron Microscopy
Specialty: Neuropathology

Vijay Vanguri, MD
Instructor of Pathology
Director, Renal Pathology
Specialty: Renal Pathology

Otto Walter, MD
Instructor of Pathology
Specialty: Surgical Pathology

James Yang, MD
Assistant Professor
Specialty: Surgical Pathology

Hongbo Yu, MD, PhD
Assistant Professor of Pathology
Director, Hematology Lab
Specialty: Hematology and Hematopathology

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Laboratories

UMass Memorial
Medical Center Laboratories
One Biotech Park, Suite 200
365 Plantation Street
Worcester, MA 01605-2376

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Photo: Kevin Vance

Our **Anatomic Pathology Department** is located in a new 35,000 square foot, state-of-the-art laboratory in the Biotech Park adjacent to the University of Massachusetts Medical School and UMass Memorial Medical Center. This laboratory processes more than 65,000 surgical pathology specimens and 80,000 cytology specimens per year.

The Anatomic Pathology Department staff includes 24 pathologists who are sub-specialists in various disciplines and who are dedicated to excellence in patient care, medical education, and research. Our pathologists are focused on providing high-quality anatomic pathology services that meet the needs of clinicians and their patients and are available for consultations with clinicians.