FISHING TO DETECT URINARY AND OTHER CANCERS: DO IMAGING SYSTEMS HELP?

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The University of Utah Department of Pathology

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Abstract

Introduction:

Urinary neoplasms are associated with a variety of chromosomal aberrations, including amplifications of chromosomes, 3, 7 and 17, and the deletion of 9p21. The Vysis UroVysionTM kit detects these aberrations by fluorescence in situ hybridization (FISH). Because the manual interpretation and documentation of UroVysion FISH is time consuming, we investigated image analysis systems designed to streamline the UroVysion FISH screening process. This study evaluated the BioView Duet imaging system as an aid to the interpretation of UroVysion FISH cases, comparing it to manual review. We additionally evaluated the BioView feature, Target FISH.

Materials and Methods:

135 consecutive UroVysion FISH cases that were received in the ARUP laboratory were screened manually as well as with the aid of a BioView Duet imaging system. Manual and BioView-aided interpretations were compared with respect to accuracy, between-run precision, and the time required to perform it.

For Target FISH, BioView was used to capture target cell images from Pap stained slides, which were then destained using acidic alcohol, before carrying out UroVysion FISH. The BioView instrument was used to relocate the original target cells, and FISH signals were evaluated.

Results:

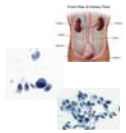
Sixty percent of the 135 cases could be interpreted as clearly positive or negative, with urine specimens showing 98.5% concordance, and non-urine specimens showing 100% concordance, between the manual and BioView-aided interpretations. Two cases judged to be BioView-positive, but manual-negative, were resolved to be positive, and thus represent manual screen false negative cases. Thirteen percent of the scanned cases displayed tetrasomy/tetraploidy or trisomy, and the remaining 27 percent of the slides were unsatisfactory for BioView interpretation because of scant cellularity or excessive clumping. The total time required for pathologist evaluation was 4 min/case with the aid of BioView, compared to 30 min/case for a manual interpretation.

For Target FISH, it was important to fully destain the slides before FISH was carried out, and some cell loss occurred during the procedure. Brightfield scans did not demonstrate the same quality as fluorescent scans, with non-cellular material and inflammatory cells often included as targets. The ability of the instrument to relocate previously scanned target cells was excellent, with target cells in near-perfect registration or located within the field of view.

Conclusion:

In this study, the BioView-aided interpretations were at least equivalent to manual interpretations, with 2 manual review false negatives cases being detected with the aid of the BioView instrument. To work optimally, cell distribution on the slide must be of high quality. The images generated are of excellent quality for archiving. The system permits interactive review of abnormal cells, as well as the ability to evaluate the same cells for brightfield cytology followed by UroVysion FISH (Target FISH).

Urothelial Carcinoma Diagnosis



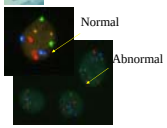
- 2006: 50,000 new cases; 12,000 deaths; high recurrence rate
- Cystoscopy (sensitive to low grade papillary tumors; may miss CIS)
- Cytology (sensitive to high grade; may miss low grade)
- Increasingly, molecular markers, including UroVysion FISH, used to aid diagnosis and monitor recurrence

Introduction to UroVysion FISH

Test kit available from Abbott/Vysis for diagnosis and monitor recurrence of bladder cancer

Multitarget, Multicolor FISH Test to Detect:

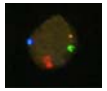
- Aneuploidy of Chromosomes:
  - 3, Red Signal
  - 7, Green Signal: (EGFR is on 7),
  - 17, Aqua Signal: (HER2/neu is on 17),
- Deletions of 9p21 (p16), Gold Signal
- DAPI, non-specific DNA or nuclear dye



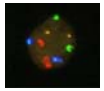
We explored the use of image processing to aid in the interpretation of UroVysion FISH

Why do we need image processing/analysis for UroVysion FISH?

- Time -- manual interpretation was requiring ~30 min/case. Would image processing produce shorter turn-around times?
- Patient care – Could image processing reduce false negative and false positive rates?
- Images for CAP archiving requirements – Could an imaging system provide quality images for archiving?
- Locations of cells – Could an imaging system track the locations of cells for re-examination?
- New tool for advancement of cytology and expanded role for trained cytotechnologists



Single focal plane – Manual Screen



Processed Image: Images in different focal planes have been captured and merged. Signal to noise ratio optimized

Imaging Systems Have Some Advantages Over the Human Eye

- Humans do not see well in dim light
- Image capture and processing can be used to adjust for variations in signal strength and background

We evaluated and selected the BioView Duet Imaging System (BioView, Ltd.) for implementation to aid in the interpretation of UroVysion FISH

BioView Duet Applications

- Automated FISH (Fluorescence)
- IHC or Stain (Brightfield)
- Target FISH (IHC/stain followed by FISH)

- Cancer
  - Hematology
  - Bladder Cancer
    - Automated UroVysion FISH (FDA approved)
    - Target FISH
  - Breast Cancer
  - Other
- Cytogenetics
- Sperm
- Rare Cells Detection

Solo Station for Reclassification

Software Classifies Cells Based on Nuclear Features and Signal Counts

Scans until preset # has been classified (often 250)

Classified:

- **Normal** (100 required to call case negative).
- **Abnormal** (cells with 2 or more of probes in excess of 2 signals). 4 or more = positive.
  - Single Gain
  - **Zero Gold** (12 or more = positive)
  - Blood
  - Suspicious blood
  - Squamous
  - Clusters

Unclassified

Expanded Roles for Cytotechnologists

- Training to perform UroVysion FISH
- Using BioView Solo, check software classification of cells
- Prepare case for evaluation by a cytopathologist and offer case interpretation

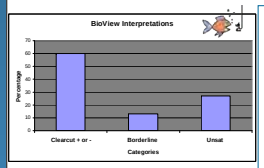
Part 1:

Validation: Manual vs BioView Aided Interpretations for 135 UroVysion FISH Cases

Materials and Methods: UroVysion FISH was carried out as described by the Abbnott/Vysis package insert. UroVysion FISH Cases (135) were initially evaluated manually as they were received in the Cytology Laboratory, then were scanned using the BioView imaging system. BioView aided interpretations were made without knowledge of the manual interpretation. The study was approved by University of Utah IRB # 00025461.

How Cases Were Interpreted:

- Positive:
  - > or = 4 cells with gains of 2 or more chromosomes (3, 7, 17)
  - > or = 12 cells with homozygous deletion of 9p21
- Negative (with 100 normal cells)
- Unsat (Manual screen & BioView scan, but uninterpretable by BioView)
- Borderline: 4 signals for each probe. Tetrasomy/tetraploidy (are they normal G2 cells or cells of tetraploid tumor?)



BioView Unsat Category:

- Many too scant or clumped for BioView Interpretation (but OK for manual screen)
- Most presumed negative cases, but did not meet 100 normal cell requirement
- Preps improving and have evaluated alternative slide preparation (Thin Prep UroCyte filter).

Concordance between Manual and BioView Aided Interpretations for Clearcut + or - Cases

Sample types

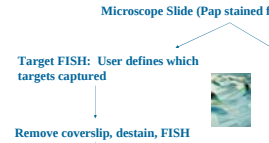
	BioView Negative	BioView Positive
Manual Negative	11 TN	
Manual Positive		9 TP

Non-Urine Cases: 100% Concordant

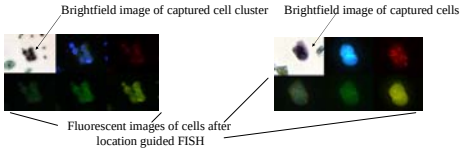
Urine Cases: 98.4% Concordant

	BioView Negative	BioView Positive
Manual Negative	47 TN	1: BioView FP **3 (resolved +, Manual FN)**
Manual Positive	1 Manual FP	9 TP

The pathologist with the aid of BioView imaging detected 3 positive cases that were missed with manual screening!



Location Guided or Target FISH



Challenges with Location Guided FISH

- Complete destaining critical to success
- Precise cell localization requires precise placement of slide on microscope stage
- Software recognition of "cells" imperfect in brightfield mode

Overall Summary and Conclusions

- BioView-Aided at least equivalent to Manual Interpretation. Valuable tool:
  - Great clinically – minimizes false negatives!
  - Interactive review of abnormal cells
  - Image enhancement of weak signals
  - Great training tool
- Pathologist time (30 min manual vs 4 min BioView); Cytotechnologist 15 + 2 min. Total 30 vs 21 min.
- Merged images of excellent quality for archiving (CAP)
- Because of high unsat rate, validated new prep (ThinPrep UroCyte Filter)
- Borderline cases – 13%. Tetrasomy/tetraploidy or trisomy.
- Precision study – excellent reproducibility

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Conflict of Interest: The authors declare that no conflict of interest relationship exists.