Bioview Duet™- Assisted Fluorescence In Situ Hybridization for Gastrointestinal Malignancy

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The University of Utah Department of Pathology

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Abstract

Introduction:

Ancillary testing using fluorescence *in situ* hybridization (FISH) may improve the accuracy of routine cytology of gastrointestinal specimens. We describe a pilot study of automated FISH using BioView Duet™-aided interpretation as an ancillary test for gastrointestinal malignancies in the cytology laboratory.

Materials and Methods:

Residual, discarded gastrointestinal cytology samples collected in either PreservCyt® or CytoLyt® were used for the validation. Slides were prepared by either a manual method or ThinPrep® UroCyte filters. UroVysion® FISH was carried out as described in the UroVysion® Bladder Cancer Kit Package Insert. UroVysion® probes detect four chromosomal aberrations by FISH, including polysomy for chromosomes 3, 7, and 17, or the homozygous deletion of 9p21. In pancreatobiliary specimens, chromosomal abnormalities have been found to fall into two groups: 1) Polysomy, in which 2 or more of the probes detect gains in chromosomes of 5 or more cells; 2) Trisomy, in which only one probe shows a single gain (usually CEP 7). The positive predictive value (PPV) of polysomy for the presence of tumor is high, whereas the PPV of trisomy is variable (from 31% to 100%) depending on the PSC status of the patient and the location of the tumor. UroVysion® FISH-stained slides were scanned using the Duet™ system, evaluated by a cytotechnologist and verified by a pathologist. Slide and stain quality, accuracy, between-run precision, and the time required to perform the test were evaluated.

Results:

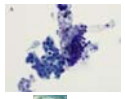
Twenty-three residual patient specimens (26 slides) were satisfactory for evaluation, with bile duct brushings the most frequent specimen type. Nine slides were FISH positive (34.6%); 14 slides were FISH negative (53.8%), and three slides were equivocal/trisomy (11.5%). The cytological interpretations for the nine positive FISH slides were a) malignancy favored (n=2, 1 case); b) suspicious for adenocarcinoma (n = 1); b) atypical (n=3), c) negative (n=2). For the one suspicious and three atypical cytology cases, the FISH-positive results should promote clinical correlation and further follow-up of the patient.

BioView-aided interpretations of UroVysion® FISH were reproducible for patient and control slides. The times required for reclassification (cytotechnologist) and verification (pathologist) were 41 and 4.9 min, respectively. Slide quality (DAPI and signal quality, cellularity and clumping) were scored from 1+ - 3+, with manual preparations scoring higher than UroCyte filter slide preparations, especially for mucoid samples that displayed interfering background fluorescence.

Conclusion:

UroVysion® FISH can be a useful ancillary method to detect malignancy in exfoliative gastrointestinal cytology cases. Duet™- aided interpretation of FISH-stained slides improved lab work flow, turn-around time, and can be incorporated into routine cytotechnologist responsibilities. Pathologist time for FISH interpretation was significantly improved.

Pancreatobiliary Carcinoma Diagnosis



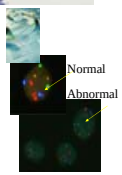
- **Biliary brush cytology: variable sensitivity for detection of malignancy (8-68%)**
- **Ancillary testing such as FISH show higher sensitivity than cytology alone**

We validated UroVysion FISH for GI Specimens Using the Duet Imaging System to Aid in Interpretation.

Introduction to UroVysion FISH for GI Specimens

UroVysion FISH kit (Abbott/Vysis): evidence supports its use for pancreatobiliary cancers: Multitarget, Multicolor FISH Test to Detect:

- Amplifications of Chromosomes:
  - 3, Red Signal
  - 7, Green Signal
  - 17, Aqua Signal:
- Deletions of 9p21 (p16), Gold Signal
- DAPI, non-specific DNA or nuclear dye



Chromosomal Abnormalities for GI Specimens Fall Primarily into 2 Groups:

- **Polysomy (2 or more probes detect gains in 5 or more cells)**
- **Trisomy (only one probe shows signal gains (usually Cep 7, less frequently Cep 3)**

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

- **Time -- manual interpretation requires > 30 min/case.**
- **Patient care – reduce false negatives and false positives?**
- **Images for CAP -- archiving requirements**
- **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 use the BioView Duet Imaging System (BioView, Ltd.) to aid in the interpretation of UroVysion FISH for GI Specimens

Duet for Scanning

Expanded Roles for Cytotechnologists

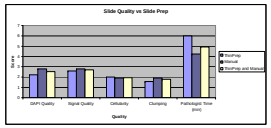
Solo Station for Reclassification by Cytotechnologists

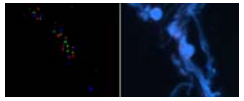
Materials and Methods: UroVysion FISH was carried out as described by the Abbott/Vysis package insert. The study was approved by University of Utah IRB # 00025364. To optimize, we compared 2 slide preparation methods:

- ThinPrep UroCyte filter
- Manual Method using 3:1 Methanol:Acetic Acid Fixation

Manual Slide Prep:

Scores higher than ThinPrep but problems with clumping and cellularity with both methods.

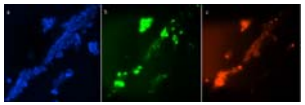




Both Preps: In a few cases mucus strands were DAPI positive with probe signals

What is in mucus strands? Varies by patient, but may include, as shown in figure below:

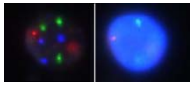
- a) DNA **(labeled with DAPI)**
- b) Mucin **(labeled with lectin from *Ulex europaeus* - FITC)**
- c) Actin **(labeled with TRITC-phalloidin)**



Preliminary studies suggest viscosity and mucin can be reduced with a wash in N-acetyl cysteine, which can reduce viscosity of mucin in vitro (Rubin, 2007). Collection of sample in CytoLyt rather than PreservCyt recommended.

How Cases Are Interpreted:

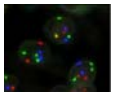
Negative: No evidence of numeric chromosomal aberrations identified.



Positive (Polysomy): Probe signal enumeration with greater than or equal to 5 cells with gains of 2 or more chromosomes.

Positive (Trisomy)

Probe signal enumeration with greater than or equal to 10 cells with gains of a single chromosome, 7 (D7Z1, Trisomy 7) or 3 (D3Z1, Trisomy 3)



Equivocal (tetrasomy): Probe signal enumeration with tetrasomy (i.e., 4 copies of chromosomes 3, 7, 17, and 9p21). Tetrasomic cells could represent cells in the G2 phase of the cell cycle or could be consistent with the presence of a tetraploid or near-tetraploid tumor. In the absence of clinically detectable tumor, close follow-up is warranted.
Equivocal (trisomy): Probe signal enumeration with <10 cells displaying trisomy.
Equivocal (polysomy): Probe signal enumeration with <5 cells displaying possible polysomy.

Unsat: (Manual screen & BioView scan, but uninterpretable by BioView)

Results

Comparison and Concordance Between UroVysion FISH & Cytology

FISH Interpretations:

- **9 slides FISH positive**
- **14 slides FISH negative**
- **3 slides equivocal**

	Negative	Positive	Equivocal Trisomy
Esophageal Brushing	2		
Jejunal Brushing		1	
Bile Duct Brushing	10	3	3
Common Hepatic Duct Brushing	1	2	
Pancreatic Duct Brushing		3	
Pancreatic Tissue	1		

Cytology Interpretations:

- **19 NMCI**
- **3 ATCP**
- **1 Suspicious**
- **2 Malignant**
- **1 Unknown**

76% Concordance between FISH and cytology

Further consideration of 2 Discrepant Cases

- **GIFD26 (positive trisomy and NMCI). 1 of 3 slides from same patient; other 2 scant but negative, and Positive trisomy)**
- **GIFD33 (1 slide each prep – case mixture of trisomy and polysomy. NMCI by cytology)**

For atypical and highly atypical cytology interpretations, positive FISH interpretations may provide support for clinical correlation and further follow-up of patient.

Reproducibility

Control Slide Reproducibility

Control	Run 1 Slide 1	Run 1 Slide 2	Run 2 Slide 1	Run 2 Slide 2
ProbeChek (+) target	Positive	Positive	Positive	Positive
ProbeChek (-) target	Negative	Negative	Negative	Negative

FISH Reproducibility for Patient Slides Positive by Cytology: 4 slides, 2 FISH/Duet Runs

Run and Scan	Slide	CEP 3 red	CEP7 green	CEP 17 blue	LSI 9p21 gold
1	GIFD23A	3.4	1.76	3.88	1.22
1	GIFD23B	3.56	3.46	3.92	0.72
2	GIFD23C	3.98	4.68	4.84	1.94
2	GIFD23D	3.2	3.7333	4.4	0.06667
Average		3.535	3.40833	4.26	0.986668
Standard Deviation		0.33121	1.21688	0.45314	0.79179

Some variation in signal counts but all 4 slides interpreted as FISH positive.

Overall Summary and Conclusions

- **UroVysion FISH is a useful ancillary method for pancreatobiliary specimens, and has been shown to be clinically more sensitive than cytological evaluation alone (Barr Fritcher, et al., 2007; Fritcher, et al., 2009; Moreno Luna and Gores, 2006; Moreno Luna, et al., 2006)**
- **Sample preparation challenging in presence of mucus – knowledge of composition may guide approaches to reducing viscosity**
  - **Mucin**
  - **DNA**
  - **Actin**
- **Manual Slide Method (fixation 3:1 Methanol:acetic acid) best with mucoid samples, but ThinPrep UroCyte filter method also may be used**
- **Reproducible**
- **Duet Imaging System of value to aid in interpretation of UroVysion FISH for pancreatobiliary specimens**
  - **Tools that enhance signal to noise ratio to improve identification of abnormal cells**
  - **Time savings for pathologists made possible by involvement of cytotechnologists**
- **Concordance between cytology and FISH was 76%**

References

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