

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

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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 UroVysion 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=3, 1 case); 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+ to 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 CAP -- 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

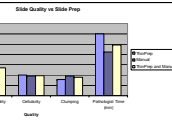
Expanded Roles for Cytotechnologists



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:

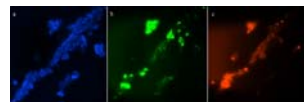
Scores higher than ThinPrep but problems with clumping and cellularity with both methods.



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

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

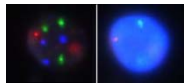
- a) DNA (labeled with DAPI)
- b) Mucin (labeled with lectin from *Ulex europaeus* - FITC)
- c) Actin (labeled with TRITC-phalloidin)



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.

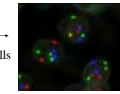
How Cases Are Interpreted:

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

	Original	Positive	Equivocal/Trisomy
Endocervical Brushing	3	0	0
Aspirated Brushing	1	0	0
Bile Duct	10	3	3
Rectal Biopsy	1	1	0
Urethral Specimen	1	0	0
Transurethral Biopsy	1	0	0

Cytology Interpretations:

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

76% Concordance between FISH and cytology

Further consideration of 2 Discrepant Cases

- G1FD26 (positive trisomy and NMCI). 1 of 3 slides from same patient; other 2 scant but negative, and Positive trisomy
- G1FD33 (1 slide each prep -- case mixture of trisomy and polysomy, NMCI by cytology)

CASE ID	Cell/Slide FISH Result	Cytology Result
G1FD23A	Positive	NMCI
G1FD23B	Positive	NMCI
G1FD23C	Positive	NMCI
G1FD23D	Positive	NMCI
G1FD23E	Positive	NMCI
G1FD23F	Positive	NMCI
G1FD23G	Positive	NMCI
G1FD23H	Positive	NMCI
G1FD23I	Positive	NMCI
G1FD23J	Positive	NMCI
G1FD23K	Positive	NMCI
G1FD23L	Positive	NMCI
G1FD23M	Positive	NMCI
G1FD23N	Positive	NMCI
G1FD23O	Positive	NMCI
G1FD23P	Positive	NMCI
G1FD23Q	Positive	NMCI
G1FD23R	Positive	NMCI
G1FD23S	Positive	NMCI
G1FD23T	Positive	NMCI
G1FD23U	Positive	NMCI
G1FD23V	Positive	NMCI
G1FD23W	Positive	NMCI
G1FD23X	Positive	NMCI
G1FD23Y	Positive	NMCI
G1FD23Z	Positive	NMCI
G1FD24A	Positive	NMCI
G1FD24B	Positive	NMCI
G1FD24C	Positive	NMCI
G1FD24D	Positive	NMCI
G1FD24E	Positive	NMCI
G1FD24F	Positive	NMCI
G1FD24G	Positive	NMCI
G1FD24H	Positive	NMCI
G1FD24I	Positive	NMCI
G1FD24J	Positive	NMCI
G1FD24K	Positive	NMCI
G1FD24L	Positive	NMCI
G1FD24M	Positive	NMCI
G1FD24N	Positive	NMCI
G1FD24O	Positive	NMCI
G1FD24P	Positive	NMCI
G1FD24Q	Positive	NMCI
G1FD24R	Positive	NMCI
G1FD24S	Positive	NMCI
G1FD24T	Positive	NMCI
G1FD24U	Positive	NMCI
G1FD24V	Positive	NMCI
G1FD24W	Positive	NMCI
G1FD24X	Positive	NMCI
G1FD24Y	Positive	NMCI
G1FD24Z	Positive	NMCI
G1FD25A	Positive	NMCI
G1FD25B	Positive	NMCI
G1FD25C	Positive	NMCI
G1FD25D	Positive	NMCI
G1FD25E	Positive	NMCI
G1FD25F	Positive	NMCI
G1FD25G	Positive	NMCI
G1FD25H	Positive	NMCI
G1FD25I	Positive	NMCI
G1FD25J	Positive	NMCI
G1FD25K	Positive	NMCI
G1FD25L	Positive	NMCI
G1FD25M	Positive	NMCI
G1FD25N	Positive	NMCI
G1FD25O	Positive	NMCI
G1FD25P	Positive	NMCI
G1FD25Q	Positive	NMCI
G1FD25R	Positive	NMCI
G1FD25S	Positive	NMCI
G1FD25T	Positive	NMCI
G1FD25U	Positive	NMCI
G1FD25V	Positive	NMCI
G1FD25W	Positive	NMCI
G1FD25X	Positive	NMCI
G1FD25Y	Positive	NMCI
G1FD25Z	Positive	NMCI
G1FD26A	Positive	NMCI
G1FD26B	Positive	NMCI
G1FD26C	Positive	NMCI
G1FD26D	Positive	NMCI
G1FD26E	Positive	NMCI
G1FD26F	Positive	NMCI
G1FD26G	Positive	NMCI
G1FD26H	Positive	NMCI
G1FD26I	Positive	NMCI
G1FD26J	Positive	NMCI
G1FD26K	Positive	NMCI
G1FD26L	Positive	NMCI
G1FD26M	Positive	NMCI
G1FD26N	Positive	NMCI
G1FD26O	Positive	NMCI
G1FD26P	Positive	NMCI
G1FD26Q	Positive	NMCI
G1FD26R	Positive	NMCI
G1FD26S	Positive	NMCI
G1FD26T	Positive	NMCI
G1FD26U	Positive	NMCI
G1FD26V	Positive	NMCI
G1FD26W	Positive	NMCI
G1FD26X	Positive	NMCI
G1FD26Y	Positive	NMCI
G1FD26Z	Positive	NMCI
G1FD27A	Positive	NMCI
G1FD27B	Positive	NMCI
G1FD27C	Positive	NMCI
G1FD27D	Positive	NMCI
G1FD27E	Positive	NMCI
G1FD27F	Positive	NMCI
G1FD27G	Positive	NMCI
G1FD27H	Positive	NMCI
G1FD27I	Positive	NMCI
G1FD27J	Positive	NMCI
G1FD27K	Positive	NMCI
G1FD27L	Positive	NMCI
G1FD27M	Positive	NMCI
G1FD27N	Positive	NMCI
G1FD27O	Positive	NMCI
G1FD27P	Positive	NMCI
G1FD27Q	Positive	NMCI
G1FD27R	Positive	NMCI
G1FD27S	Positive	NMCI
G1FD27T	Positive	NMCI
G1FD27U	Positive	NMCI
G1FD27V	Positive	NMCI
G1FD27W	Positive	NMCI
G1FD27X	Positive	NMCI
G1FD27Y	Positive	NMCI
G1FD27Z	Positive	NMCI

Reproducibility

Control Slide Reproducibility

Control	Run 1 Slide 1	Run 1 Slide 2	Run 2 Slide 1	Run 2 Slide 2
ProbeCheck (+) target	Positive	Positive	Positive	Positive
ProbeCheck (-) 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	G1FD23A	3.4	1.76	3.88	1.22
1	G1FD23B	3.56	3.46	3.92	0.72
2	G1FD23C	3.98	4.68	4.84	1.94
2	G1FD23D	3.2	3.7330	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 mucin -- 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

- Barr Fritcher, et al. Am. J. Clin. Pathol. 2007; 128:272-279
- Fritcher EG et al. Gastroenterology 2009; 136(7):2180-2186
- Halling KC, Kipp BR, Human Pathol 2007; 38(8):1137-1144
- Moreno Luna LE, Gores GJ, Liver Transpl 2006; 12(11 Suppl 2):S15-19
- Moreno Luna et al., Gastroenterology 2006; 131(4); 1064-1072
- Rubin BK Respiratory Care 2007; 52(7):859-865

Acknowledgments

- Matt Riding, CT (ASCP), Supervisor ARUP Cytology, & ARUP Cytology Staff
- Bryan Lindsey, Gary Isom, & ARUP Hematopathology Staff
- BioView, Ltd. Support Staff. Some poster images are from the BioView Ltd website: <http://www.bioview.co.uk/HTML/Home.aspx>
- This study was supported by the ARUP Institute for Clinical and Experimental Pathology®

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