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

G. D. Smith1 and J. S. Bentz1,2

1ARUP Institute for Clinical and Experimental Pathology®, ARUP Laboratories, Salt Lake City, UT, USA 2Department of Pathology, University of Utah, Salt Lake City, Utah, USA

Abstract

Background: Cytomorphological analysis of cytology specimens is the gold standard for the diagnosis of urothelial carcinoma. However, intra- and interobserver variability has been noted in this analysis. UroVysion is a fluorescent in situ hybridization (FISH) test that can detect specific genetic abnormalities to help in the differential diagnosis of urinary and other cancers. We evaluated the BioView Duet Imaging System in an effort to improve the accuracy of cell interpretation.

Methods: UroVysion FISH was carried out as described by the Abbott/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 # 0002561.

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

Conclusion: The use of BioView Duet Imaging System allows for improved detection of UroVysion FISH positivity.

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
- Pathologists time (30 min manual vs 4 min BioView); Cytologist time 15 ± 2 min. Total 30 ± 21 min.
- Merged images of excellent quality for archiving (CAP)
- Because of high-sensitivity, validated new prep (ThinPrep UroCyte Filter):
- Borderline cases – 13%. Tetrynomyelopy or mytumy.
- Precision study – excellent reproducibility

Acknowledgments

Matt Rabung, CT (ASCAP), Supervisor ARUP Cytology
ARUP Cytology Staff
Bryan Lindsey, Gary J. Williams, ARUP Hematopathology Staff
This study was supported in part by the ARUP Institute for Clinical and Experimental Pathology.

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

BioView Duet Applications

- Automated FISH (Fluorescence)
- IHC or Stain (QuickFISH)
- Target FISH (IHC/stain followed by FISH)
- Multiple FISH
- Intercalated IHC
- Fluorescence
- Spectroscopy
- Rare Cell Detection

Solo Station for Reclassification

Software Classifies Cells Based on Nuclear Features and Signal Counts

- Normal (10 signals per cell [see table])
- Abnormal (cells with 2 or more of probes in excess of 2 signals; + = more = positive; Single bar)
- Zero Gold (12 or less = positive)
- Abnormal with 2 or more of probes in excess of 2 signals; + = more + positive
- Single bar
- Suggestive Signal
- Unclassified

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

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-Urine Cases: 98% Concordant</td>
<td>41</td>
<td>9 TP</td>
<td>2</td>
<td>17</td>
</tr>
</tbody>
</table>

Microscope Slide (Pop stained for brightfield)

Target FISH: Use defines which target captured

Bleacher Cancer Target FISH: BioView Automated Scan defines targets

Remove coverslip, destain, FISH

Check FISH interactively on BioView

Location Guided or Target FISH

Reclassify and evaluate

Fluorescent images of cells after location guided FISH

Brightfield image of captured cell cluster

Brightfield image of captured cells

Challenges with Location Guided FISH

Complete distracting critical to success

Precise cell localization requires precise placement of slide on microscope stage

Software recognition of "cells" imaged in brightfield mode

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?
- Location of cells – Could an imaging system trace the locations of cells for re-examination?
- New tool for advancement of cytology and expanded role for trained cytomorphologists

Images for FISH and Cytology

- Single focal plane – Manual Screen
- Location Guided or Target FISH
- Check FISH interactively

Location Guided FISH: Materials and Methods

- UroVysion FISH was carried out as described by the Abbott/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.
- How Cases Were Interpreted:
  - For Target FISH, BioView was used to capture target cell images from Pap stained slides, which were then destained and before carrying out UroVysion FISH.
  - The BioView instrument was used to relocate the original target cells, and FISH signals were evaluated.
  - Manual interpretation was performed on Pap stained slides, which were then destained and before carrying out UroVysion FISH.
  - The BioView instrument was used to relocate the original target cells, and FISH signals were evaluated.

Results:

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

Conclusion:

- The use of BioView Duet Imaging System allows for improved detection of UroVysion FISH positivity.

Overall Summary and Conclusions

BioView FISH test 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
- Pathologists time (30 min manual vs 4 min BioView); Cytologist time 15 ± 2 min. Total 30 ± 21 min.
- Merged images of excellent quality for archiving (CAP)
- Because of high-sensitivity, validated new prep (ThinPrep UroCyte Filter)
- Borderline cases – 13%. Tetrynomyelopy or mytumy.
- Precision study – excellent reproducibility

Acknowledgments

Matt Rabung, CT (ASCAP), Supervisor ARUP Cytology
ARUP Cytology Staff
Bryan Lindsey, Gary J. Williams, ARUP Hematopathology Staff
This study was supported in part by the ARUP Institute for Clinical and Experimental Pathology.

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

Fluorescent images of cells after location guided FISH

Brightfield image of captured cell cluster

Brightfield image of captured cells

Challenges with Location Guided FISH

Complete distracting critical to success

Precise cell localization requires precise placement of slide on microscope stage

Software recognition of “cells” imaged in brightfield mode

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?
- Location of cells – Could an imaging system trace the locations of cells for re-examination?
- New tool for advancement of cytology and expanded role for trained cytomorphologists