# Target UroVysion™ FISH for Urinary Specimens Using the Duet™ Imaging System

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*An enterprise of the University of Utah and its Department of Pathology*

UNIVERSITY OF UTAH SCHOOL OF MEDICINE | Department of Pathology


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## Abstract


**Background:** Cytology has a poor sensitivity for urothelial carcinoma, and molecular tests such as UroVysion FISH may aid in interpretation. Target (or Location Guided) FISH is an ideal tool for combining morphology (stained or immunolabeled cells) with FISH. Studies by Daniely et al, provide clinical evidence that the combined analysis of morphology and FISH has high sensitivity and negative predictive value for the detection of bladder cancer.

**Design:** The BioView Duet imaging system was used to identify abnormal-appearing cells by cytology followed by UroVysion FISH. A preliminary study using Pap-stained slides followed by UroVysion FISH showed problems with cell recovery and stain interference with subsequent FISH. To reduce cell loss and stain interference, this study included 19 slides that were stained with May-Grunwald/Giemsa and not coverslipped. Target cells were identified by brightfield microscopy, followed by slide de-staining and UroVysion FISH, to detect amplifications of chromosomes 3, 7, and 17, and deletions of 9p21. Target cells were then re-located using Duet software and fluorescence microscopy, and probe signals were scored for cell recovery, target cell re-location accuracy, FISH signal strength, reproducibility, and correlation with cytology results.

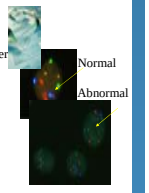
**Result:** Brightfield, DAPI, and signal quality scored 2.95, 2.95, and 3.0 (possible 3.0), and reproducibility was 100%. Target cell re-location was highly accurate with target cells re-located to the microscope field center in all but one case, for which 86% of the target cells re-located to the field center. Average target cell recovery was 78%, with >85% recovery for slides showing no scrape marks by fluorescence microscopy. Other slides with visible scrape marks had a 63% cell recovery, with damage presumed to occur during coverslip removal after FISH hybridization. Correlation with urine cytology was 92.8%. One of three atypical cytology cases was FISH positive, which may provide support for clinical correlation and further patient follow-up.

**Conclusion:** The tools provided by the Duet system for Target FISH have potential for reflex testing, combined immunocytochemistry/FISH, and research applications. In our experience, setting up a limited fluorescence scan with targets selected interactively rather than by automated scan, appears to be the best approach for 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




UroVysion FISH kit (Abbott/Vysis): Test kit available from Abbott/Vysis for diagnosis and monitor recurrence of bladder cancer
Multitarget, Multicolor FISH Test to Detect:
- Amplifications of Chromosomes (normal cells should have 2 signals for each probe):
  - 3, Red Signal
  - 7, Green Signal
  - 17, Aqua Signal:
- Deletions of 9p21 (p16), Gold Signal
- DAPI, non-specific DNA or nuclear dye

Interpretations:
Negative: 2 signals each probe
Positive:
4 or more cells with >2 signals for 2 or more probes
12 or more cells with homozygous deletion of 9p21

### What is Target FISH and how might it be useful to urine cytology?

With Target (or Location Guided FISH), stained or immunolabeled slides are examined to identify target cells of interest. Following FISH on the same slide, the target cells are re-examined by fluorescence microscopy.

- Diagnostic interpretation using a combination of morphology with Giemsa or Pap stained slides, plus UroVysion FISH on the same slides has higher sensitivity than either method alone. Can be used for reflex testing.
- An antibody such as CK7 can be used to clearly distinguish and pre-select urothelial cells from contaminating cervical cells for FISH interpretation.
- Another independent marker may be used to identify abnormal urothelial cells before FISH interpretation.

### Materials and Methods:

*Overview:* With Location Guided or Target FISH:

1. Slides are first stained with May-Grunwald/Giemsa (or Immunolabeled)
2. Abnormal cells are selected (coordinates "remembered") using the BioView Duet imaging system by one of 2 ways:
   a. an automatic scan and reclassification by a cytotechnologist, OR
   b. capturing target cells using interactive mode
3. The slides are then destained before carrying out UroVysion FISH
4. Using the Duet imager, the abnormal cells selected under brightfield are re-examined by fluorescence microscopy.

**We use the Duet Imaging System to scan and aid in the interpretation of FISH and Target FISH – Reasons:**

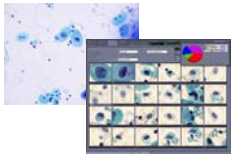
- Humans do not see well in dim light
- Image capture/processing can adjust for variations in signal strength and background
- System remembers cell locations for brightfield and fluorescence imaging

*Expanded Roles for Cytotechnologists*

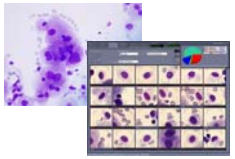
Duet Scanning Station

Solo Station for Reclassification by Cytotechnologists

### Examples of DiffQuik or Pap Stained Slides Followed by UroVysion FISH

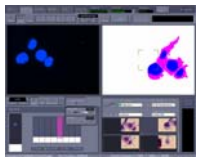


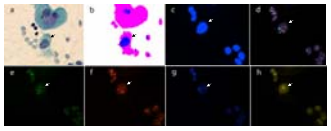
Pap Stained Slide and Duet Gallery of Brightfield Scan (Bladder Washing)



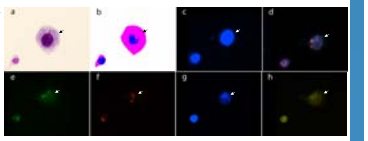
DiffQuik Stained Slide and Duet Gallery of Brightfield Scan (Bladder Washing)

Screen Capture of Duet Interface: Target FISH of Pap Stained Slide (Right Renal Wash)





Target FISH of bladder washing specimen: a) Pap stained cells; b) Duet representation of cells; c) DAPI; d) combined image of red, green, and aqua signals; e) CEP3 Spectrum Red; f) CEP7 SpectrumGreen; g) CEP 17 SpectrumAqua; h) LSI 9p21 Spectrum Gold. Arrow indicates cell with 4 signals for each probe.



Target FISH of right renal wash specimen: a) Diff Quik stained cells; b) Duet representation of cells; c) DAPI; d) combined image of red, green, and aqua signals; e) CEP3 Spectrum Red; f) CEP7 SpectrumGreen; g) CEP 17 SpectrumAqua; h) LSI 9p21 Spectrum Gold. Arrow indicates cell with 4 signals for each probe.

## Validation Study

*Description* We carried out a validation study to use Target FISH for reflex testing of cytology followed by UroVysion FISH. UroVysion FISH was carried out as described by the Abbott/Vysis package insert.

- 19 slides from 18 residual urine samples (15 males, 3 females; avg. age 62
- Slides stained by May-Grunwald Giemsa (preliminary studies suggested Pap interference with FISH and variable but occasionally up to 45% cell loss)
- Target cells selected using BioView Duet by automated scan or using Interactive mode
- Slides destained, followed by UroVysion FISH
- Duet-aided interpretation
- Validation evaluated 1) signal, DAPI, and brightfield image quality; 2) % cell recovery following FISH; 3) target cell relocation accuracy; 4) reproducibility; 5) Target FISH correlation with original cytology.

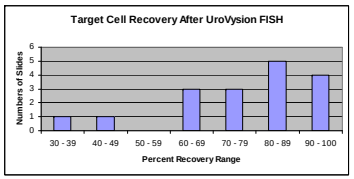
## Validation Results Summary

- **92.3% concordance between cytology and Target FISH when cytology results were definitively normal or malignant**
- **Brightfield, DAPI, and signal image quality high (scores 2.95 (2.63), 2.95 (2.94), and 3.0 (2.86), respectively when judged by 1 pathologist (or average of 2 pathologist and cytotechnologist)**
- **Cell recovery high (85% avg. for slides in which coverslip carefully removed following FISH hybridization)**
- **Target cell relocation to center of field 100% for all but 1 case (86% of cells in center)**
- **Interactive selection of target cells is currently superior to selection by automated scan. The software selects too many objects as targets that are noncellular or not cells of interest. Interactive selection required an average of 23 minutes for 40 target cells. Reclassification of automated scan required an average of 1 hr 45 minutes.**
- **Time for pathologist interpretation varied depending on experience but averaged 10.3 min. for 1 pathologist (or 6.8 min for 2 pathologists).**

*Validation Results for 19 Sample and Control Slides*

| Code # | FISH Interpretation | Cytology | % Recovery | Target Cell Re-location Accuracy (% in center of field) |
|---|---|---|---|---|
| UVTF30 | Negative (Test-plate) | NDCT | 94 | 100 |
| UVTF31 | Negative | NDCT | 96.3 | 100 |
| UVTF32 | Negative | NDCT | 78 | 100 |
| UVTF33 | Positive Polysomy | ATCP | 100 | 100 |
| UVTF34 | Positive Polysomy | MCUC | 72.5 | 100 |
| UVTF34B | Positive Polysomy | MCUC | 91.4 | 100 |
| Comb1091209SA | Negative | NDCT | 80 (scrape marks) | 100 |
| UVTF35 | Negative | NDCT w/mi inflammation | 64.7 (scrape marks) | 100 |
| UVTF37 | Negative | NDCT | 45 (scrape marks) | 100 |
| UVTF38 | Negative | NDCT w/mi cellularity | 57.9 (scrape marks) | 100 |
| UVTF39 | Scant but negative | MCUC | 85.3 (scrape marks) | 100 |
| UVTF40 | Negative | NDCT | 66.7 (scrape marks) | 85.7 perfect, 100 in field |
| UVTF41 | Negative | ATPS | 90 | 100 |
| UVTF42 | Scant but negative | NDCT (positive) | 75 | 100 |
| UVTF44 | Negative | NDCT | 84.6 | 100 |
| UVTF46 | Negative | ATCP | 94 | 100 |
| UVTF47 | Negative | NDCT | 61.5 | 100 |
| UVTF34TF scan | Negative | Not available | Not available | Not available |
| UVTF34TF scan | Negative | Not available | Not available | Not available |
| | | | | |
| ProbeCheck Pos target | Positive | MCP | | |
| ProbeCheck Neg target | Negative | NDCT | | |

*Target Cell Recovery*




*Care must be taken during coverslip removal to ensure cell recovery. Slides with low recovery percentage had visible scrape marks by fluorescence microscopy*

*Reproducibility for Validation Study*

One residual sample had sufficient sample to prepare 2 slides, each of which was carried through the Target FISH procedure on separate occasions, and evaluated.

| Code # | FISH Interpretation | Cytology | Cell Recovery | Target Cell Relocation Accuracy (% in center of | Brightfield Quality | Signal Strength | DAPI Quality |
|---|---|---|---|---|---|---|---|
| UVTF34 | Positive Polysomy | MCUC | 72.50% | 100 | 3 | 3 | 3 |
| UVTF34B | Positive Polysomy | MCUC | 91.40% | 100 | 3 | 3 | 3 |

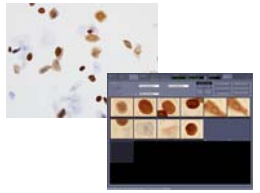
## Pilot Study for Target UroVysion FISH Using CK7 Antibody to Preselect Urothelial Cells

*Principle:* CK7 Immunocytochemistry can be used to distinguish expressing urothelial cells from contaminating cervical epithelial cells, which do not express CK7 (Moll, et al., 1982; Pajor, et al., 2008). The procedure is:

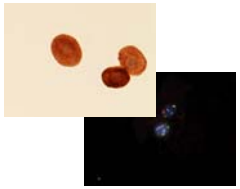
- Immunolabel urine cytology slides with CK7 antibody
- Scan slides by brightfield microscopy using Duet imaging system.
- Segregate CK7-expressing urothelial cells into a single class during reclassification.
- Destain slide and carry out UroVysion FISH.
- Carry out limited fluorescent scan of only urothelial cells.

*Challenges:*

- Currently interacting with BioView personnel to improve software capabilities to better recognize immunolabeled cells.
- Important to lightly immunolabel the cells or fully de-stain the slides before UroVysion FISH.



CK7-labeled Slide and Duet Gallery of Brightfield Scan



CK7-labeled Cells and UroVysion FISH of Previously CK7-labeled Cells

## Overall Summary and Conclusions

- **Target UroVysion FISH is a useful ancillary method for urinary specimens, particularly for reflex testing or when only stained slides are available. Cell morphology can be directly correlated with FISH results using the Duet system.**
- **It is important to fully de-stain slides before carrying out UroVysion FISH; Pap stain may interfere with FISH hybridization.**
- **Signal, DAPI, and brightfield image qualities all good (scores 2.95, 2.95 or 3 out of 3)**
- **Cell recovery is high when care is taken to carefully remove the coverslip following FISH hybridization.**
- **Target cell relocation using the Duet system is accurate.**
- **Reproducible**
- **Concordance between cytology and Target FISH was 92.3% for those cases with definitive cytology interpretations (malignant or normal)**
- **Currently, selection of target cells using the Interactive mode is best.**

### References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- Halling KC, et al. *J Urol.* 2000; 164:1768-1775.
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- Pajor G, et al. *Cytometry A* 2008; 73(3):259-265.


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