

FOR PATHOLOGISTS

Reading UroVysion®

UroVysion Bladder Cancer Kit

Pathologists can turn to CYNOGEN
for UroVysion training.

CYNOGEN provides UroVysion training to support accurate reading of UroVysion results. In addition, CYNONE scientists and technologists are always available for consultation with customers. The following information is provided for easy reference.

Probe Evaluation UroVysion® Recommendations

- Adjusting the depth of focus and become familiar with the size and shape of the target signals and noise (debris)
- Beginning the analysis in the upper left quadrant of the target area
- Scanning fields from left to right and top to bottom

Cell Selection Criteria

Use the following criteria to select cells suspicious for malignancy (morphologically abnormal).

Normal cells do not count:

Single cell



Two overlapping cells



Suspicious for malignancy do count:

Irregular nuclear shape



Patchy DAPI staining



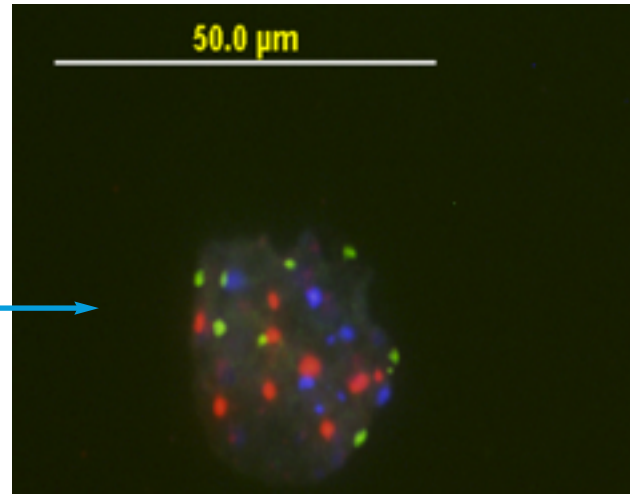
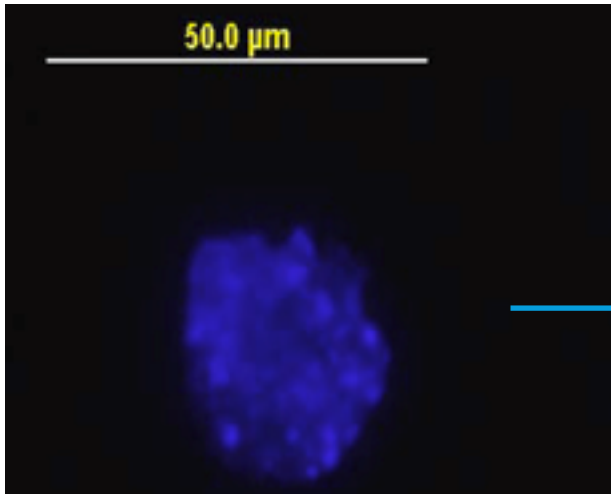
Cell Clusters



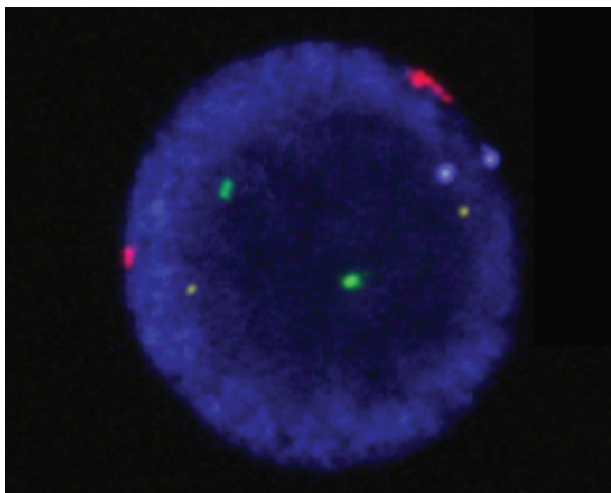
Scanning for Morphologically Abnormal Nuclei Helps Speed Microscopic Analysis for FISH

- The pathologist scans for cells with morphological features suggestive of malignancy under the DAPI Filter.
- When an abnormal cell is found, evaluate the signals under the Green/Red, Aqua, and Gold filters.

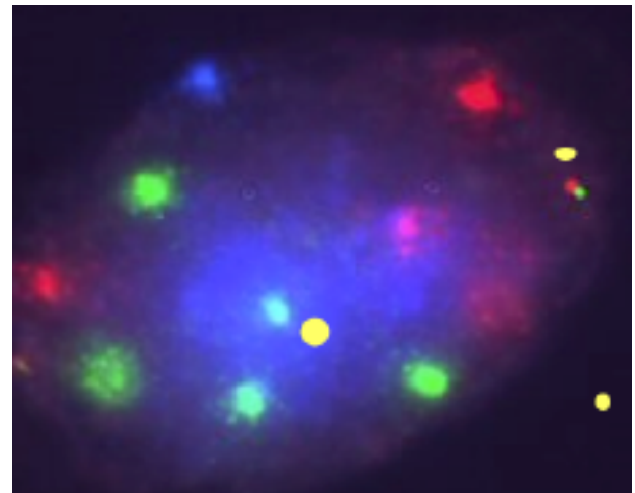
Images are examples of UroVysion results; individual results may vary.



Scanning for Morphologically Abnormal Cells



Normal Cell



Abnormal Cell

Counting Abnormal FISH Results

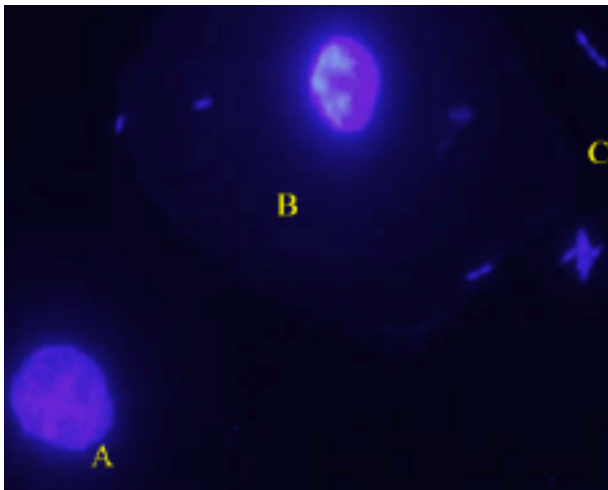
- Record the total number of morphologically abnormal cells (diploid and abnormal). Continue this process until at least 25 morphologically abnormal cells have been analyzed. If few morphologically abnormal cells are present, select cells with the largest nuclei.
- If any of the following criteria have been met, STOP the analysis:
 - ≥ 4 of the 25 cells show gains for 2 or more chromosomes (3, 7 or 17) in the same cell, **or**
 - ≥ 12 of the 25 cells have zero 9p21 signals
 - Otherwise, continue analysis until 4 cells with gain for multiple chromosomes have been detected, or 12 cells with zero 9p21 signals have been detected, or the entire sample has been analyzed.

Sample Signal Enumeration Scoring Sheet

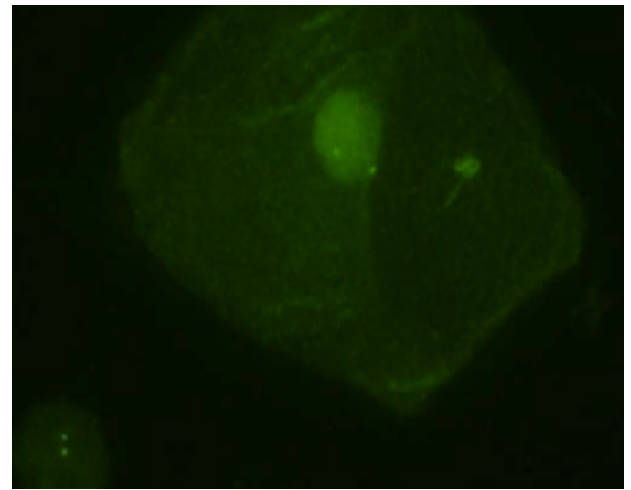
Nuclei #	CEP 3 (red)	CEP 7 (green)	CEP 17 (aqua)	LSI 9p21 (gold)	Aneuploidy +CEP	Deletion +LSI
1	3	3	2	0	X	X
2	3	4	2	1	X	
3	4	4	3	0	X	X
4	2	2	2	2		
5	2	3	3	0	X	X
6	2	2	2	2		
7	2	5	2	2		
8	4	5	3	1	X	
25	2	2	2	0		X

The package insert requires that a minimum of 25 abnormal cells be evaluated per specimen slide.

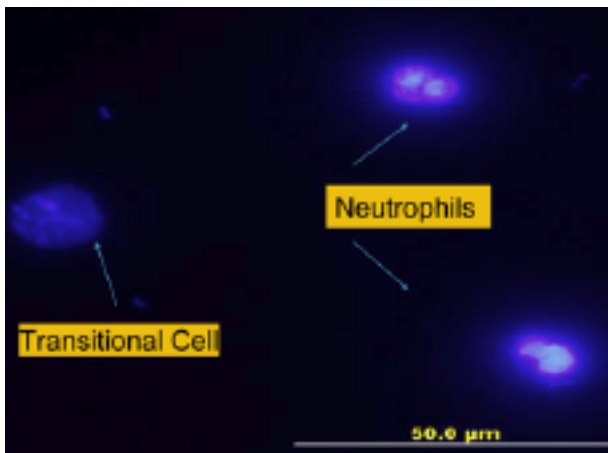
Cell Identification – Squamous Cells



A) Transitional cell B) Squamous cell C) Bacteria



Cell Identification



Images are examples of UroVysion results; individual results may vary.

Single Color Signal Counting Guide

Don't count, skip over.
This could be two cells with one signal each or one twisted nucleus.



Count as 2 signals: one is very compact, the other is diffuse.



Don't count, skip over. Observer cannot determine which cell contains the signals.



Count as 2 signals. One signal is split.



Count as three signals.



Count as four signals.



Count as three signals. One is split.



Dual Color Signal Counting Guide

Count as one red signal and one green signal. The red signal is diffuse.



Count as one red signal and two green signals. One green signal is split and the red signal is split.



Count as two red signals and one green signal.



Count as three red signals and one green signal.



Count as four red signals and two green signals.



Don't count - nuclei are overlapping and all areas of both nuclei are not visible.



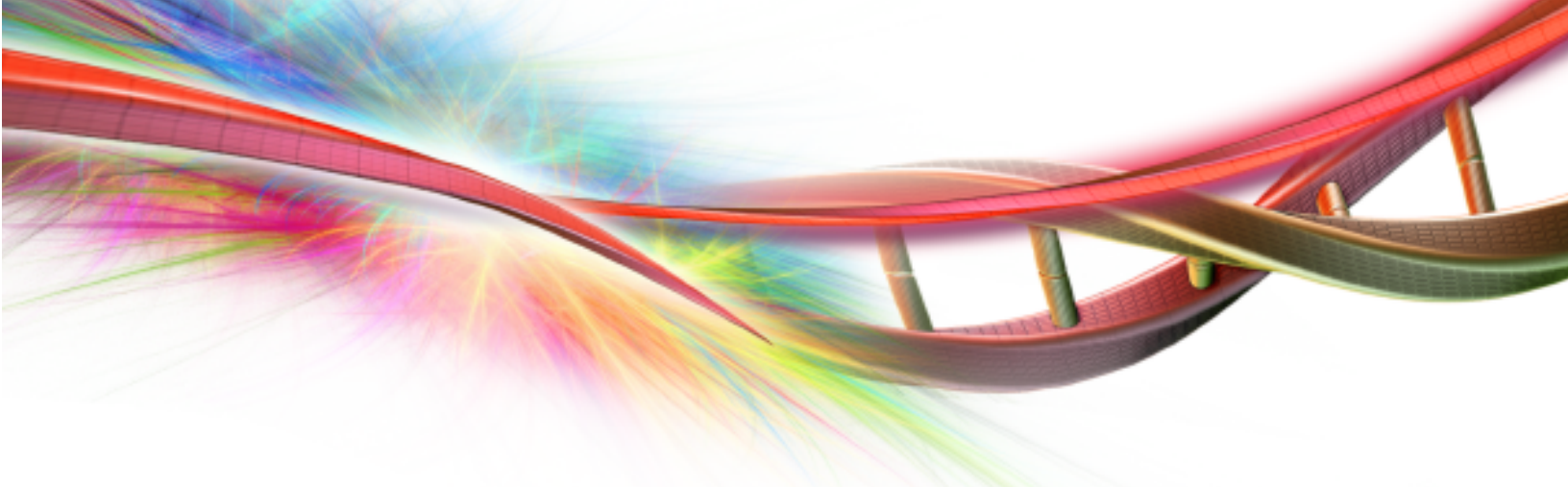
Don't count. Nuclei are too close together to determine boundaries.



Count as one red signal and one green signal. The red signal is split.



FOR *IN VITRO* DIAGNOSTIC USE



UroVysion® Bladder Cancer Kit is supported by years of scientific data allowing urologists to make evidence-based decisions regarding bladder cancer patients. UroVysion Bladder Cancer Kit is the first FDA-approved urine-based molecular diagnostic assay that aids in the initial diagnosis of bladder cancer in patients presenting with hematuria, while also monitoring for disease recurrence in previously diagnosed patients.

Clinician Benefits

- Detects chromosomal abnormalities associated with the development and recurrence of bladder cancer
- Delivers positive/negative results in suspicious cystoscopy or atypical cytology cases
- Shows 100% sensitivity among the most severe tumors (T2 and Tis)
- Performs without interference in the presence of substances such as BCG, Mytomycin C, and Thiotepa in urine samples

Intended Use

The UroVysion Bladder Cancer Kit (UroVysion Kit) is designed to detect aneuploidy for chromosomes 3, 7, and 17, and loss of the 9p21 locus via fluorescence in situ hybridization (FISH) in urine specimens from persons with hematuria suspected of having bladder cancer. Results from the UroVysion Kit are intended for use, in conjunction with and not in lieu of current standard diagnostic procedures, as an aid for initial diagnosis of bladder carcinoma in patients with hematuria, and subsequent monitoring for tumor recurrence in patients previously diagnosed with bladder cancer.

Warnings and Limitations

1. The UroVysion Kit has been optimized for identifying and quantitating chromosomes 3, 7, and 17, and locus 9p21 in human urine specimens.
2. The performance of the UroVysion Kit was validated using the procedures provided in the package insert only. Modifications to these procedures may alter the performance of the assay.
3. The clinical interpretation of any test results should be evaluated within the context of the patient's medical history and other diagnostic laboratory test results.
4. UroVysion assay results may not be informative if the specimen quality and/or specimen slide preparation is inadequate, e.g., the presence of excessive granulocytes or massive bacteruria.
5. Technologists performing the UroVysion signal enumeration must be capable of visually distinguishing between the red and green signals.
6. Positive UroVysion results in the absence of other signs or symptoms of bladder cancer recurrence may be evidence of other urinary tract related cancers, e.g., ureter, urethra, renal, and/or prostate in males, and further patient follow-up is justified. In a study conducted on patients with hematuria, 3 patients whose initial bladder cystoscopy was negative were subsequently diagnosed with renal cancer within 6 months of this initial study visit. All 3 of these cases were positive by UroVysion.
7. If UroVysion results are negative but standard clinical or diagnostic tests (e.g., cytology, cystoscopy) are positive, the standard procedures take precedence over the UroVysion test. Although the UroVysion test Kit was designed to detect genetic changes associated with most bladder cancers, there will be some bladder cancers whose genetic changes cannot be detected by the UroVysion test.
8. Ta stage solitary tumors smaller than 5mm could not be detected by UroVysion FISH. UroVysion FISH results are dependent on the amount of tumor cells that are deposited on the slide.

FOR IN VITRO DIAGNOSTIC USE

Caution

United States Federal law restricts this device to sale and distribution to, or on the order of, a physician or a clinical laboratory; use is restricted to, by, or on the order of a physician.

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