UroVysion Bladder Cancer Kit

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REF 02J27

30-608385/R6

UroVysion

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Key to Symbols Used

	Manufacturer
REF	Reference Number
IVD	In Vitro Diagnostic Medical Device
Σ	Contains sufficient for <n> tests
	Temperature Limitation
	Biological Risks
	Danger
	Danger
	Danger
	Consult instructions for use
	Use By
EC REP	Authorized Representative in the European Community

UROVYSION BLADDER CANCER KIT

(Part No. 30-161070, List No. 02J27-025;
Part No. 32-161070, List No. 02J27-020;
Part No. 36-161070, List No. 02J27-095;
Part No. 37-161070, List No. 02J27-099)

PROPRIETARY NAME

UroVysion Bladder Cancer Kit

COMMON OR USUAL NAME

Fluorescence in situ hybridization (FISH) reagents

INTENDED USE

The UroVysion Bladder Cancer Kit (UroVysion Kit) is designed to detect aneuploidy for chromosomes 3, 7, 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.

SUMMARY AND EXPLANATION

An estimated 70,530 new cases of urinary bladder cancer will be diagnosed in the United States (52,810 men and 18,170 women) in 2010.[1] Bladder cancer, the fourth most common cancer, is 3 times more common in men than women in the United States.[1] During the same period, approximately 14,680 deaths (10,410 men and 4,270 woman) from bladder cancer are anticipated.[1] Bladder cancers are rarely diagnosed in individuals younger than 40 years.[1] Because the median age of diagnosis is 65 years, medical comorbidities are a frequent consideration in patient management.[1] Ninety percent of bladder cancer cases are classified as transitional cell carcinomas (TCC), while the remaining 10% are predominantly squamous cell or adenocarcinomas.[2] There are 4 clinically relevant subgroups of TCC, as defined by pathologic staging: carcinoma in situ (pTIS), non-invasive papillary TCC (pTa), minimally invasive TCC (pT1), and muscle invasive tumors (pT2-pT4). Each subgroup differs in clinical outcome.[2,3] At presentation, 75% of tumors are "superficial" (ie, pTa, pT1 or pTIS), of which 50 to 80% will have 1 or several recurrences, and 15 to 25% will progress to invasive tumors.[4] For this reason, patients with "superficial" bladder cancer are regularly monitored for tumor recurrence and progression with cystoscopy and sometimes urine cytology. Cystoscopy examination of the bladder, and often urine cytology, are also standard care for patients > 40 years of age and presenting with hematuria.[5]

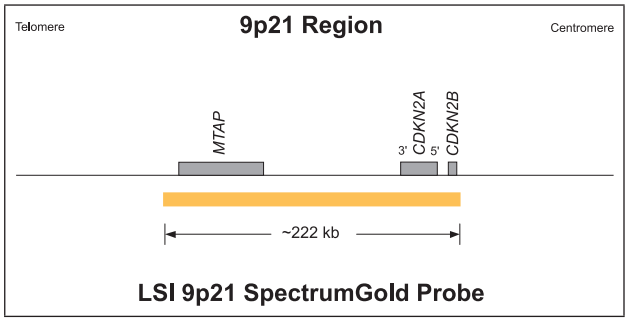
A number of studies, however, have demonstrated that urine cytology has a disappointingly low sensitivity for bladder cancer detection[6,7] and improved laboratory tests for bladder cancer detection are needed. Recent studies have demonstrated that FISH analysis for aneuploidy of specific chromosomes may be useful to aid in the detection of bladder cancer.[4,8-21]

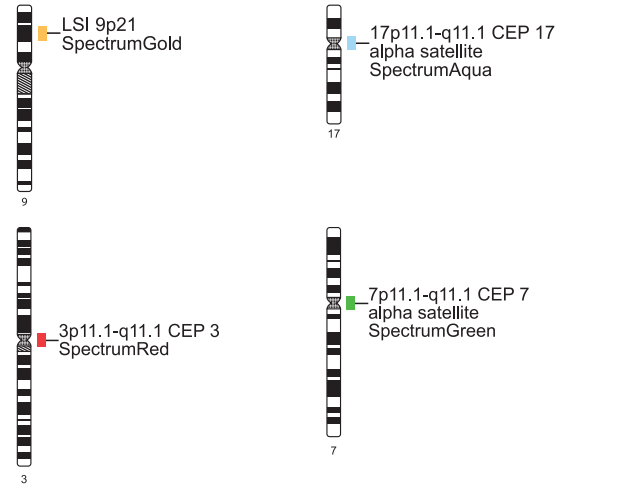
PRINCIPLES OF THE PROCEDURE

In situ hybridization is a technique that allows the visualization of specific nucleic acid sequences within a cellular preparation. Specifically, DNA FISH involves the precise annealing of a single stranded, fluorescently labeled DNA probe to complementary target sequences. The hybridization of the probe with the cellular DNA site is visible by direct detection using fluorescence microscopy.

The UroVysion probes are fluorescently labeled nucleic acid probes for use in in situ hybridization assays on urine specimens fixed on slides. The UroVysion kit consists of a 4-color, 4-probe mixture of DNA probe sequences homologous to specific regions on chromosomes 3, 7, 9, and 17. The UroVysion probe mixture consists of Chromosome Enumeration Probe (CEP) 3 SpectrumRed, CEP 7 SpectrumGreen, CEP 17 SpectrumAqua and Locus Specific Identifier (LSI) 9p21 SpectrumGold. The probes are premixed and predenatured in hybridization buffer for ease of use. Unlabeled blocking DNA is also included with the probes to suppress sequences contained within the target loci that are common to other chromosomes. When hybridized and visualized, these probes provide information on chromosome copy number for chromosome ploidy enumeration. This UroVysion Kit is designed for the detection and quantification of chromosomes 3, 7, and 17, and the 9p21 locus in human urine specimens by FISH.

Cells recovered from urine pellets are fixed on slides. The DNA is denatured to its single stranded form and subsequently allowed to hybridize with the UroVysion probes. Following hybridization, the unbound probe is removed by a series of washes, and the nuclei are counterstained with DAPI (4,6 diamidino-2-phenylindole), a DNA-specific stain that fluoresces blue. Hybridization of the UroVysion probes is viewed using a fluorescence microscope equipped with appropriate excitation and emission filters allowing visualization of the intense red, green, aqua, and gold fluorescent signals. Enumeration of CEP 3, 7, and 17, and LSI 9p21 signals is conducted by microscopic examination of the nucleus.





REAGENTS AND INSTRUMENTS

Materials Provided

This kit contains sufficient reagents to process approximately 20 or 100 assays (dependent on part number). An assay is defined as one 6 mm diameter round target area.

1) UroVysion DNA Probe Mixture
   - Part No.: 30-171070 (20 Tests); 36-171070 (100 Tests)
   - Quantity: 60 µL (20 Tests); 300 µL (100 Tests)
   - Storage: –20°C in the dark
   - Composition: Fluorophore-labeled DNA probes for chromosomes 3, 7, and 17, and locus 9p21 in hybridization buffer. The hybridization buffer is made up of dextran sulfate, formamide and SSC.

2) DAPI II Counterstain
   - Part No.: 30-804841 (20 Tests); 30-804941 (100 Tests)
   - Quantity: 300 µL (20 Tests); 1000 µL (100 Tests)
   - Storage: –20°C in the dark
   - Composition: 125 ng/mL DAPI (4,6-diamidino-2-phenylindole) in 1,4-phenylenediamine, glycerol, and buffer

3) NP-40
   - Part No.: 30-804820
   - Quantity: 4 mL (2×2 mL)
   - Storage: –20°C to 25°C
   - Composition: NP-40 (non-ionic detergent)

4) 20X SSC
   - Part No.: 30-805850
   - Quantity: 66 g for up to 250 mL of 20X SSC solution
   - Storage: –20°C to 25°C
   - Composition: sodium chloride and sodium citrate

Storage and Handling

Store the unopened UroVysion Kit as a unit at –20°C, protected from light and humidity. The 20X SSC and NP-40 may be stored separately at room temperature. Expiration dates for each of the unopened components are indicated on the individual component labels. These storage conditions apply to both opened and unopened components.

Exposure to light, heat, or humidity may affect the shelf life of some of the kit components and should be avoided. Components stored under conditions other than those stated on the labels may not perform properly and may adversely affect the assay results.

Materials Required But Not Provided

Laboratory Reagents

NOTE: Where storage conditions are not listed in this insert or the product label store reagent per vendor recommendations.

- ProbeChek UroVysion Bladder Cancer Kit Control Slides Part No. 30-805070/List No. 02J27-011 and Part No. 32-805070/List No. 02J27-010. Three glass microscope slides containing both a positive control and a negative control on the same slide (ie, 2 target areas per slide—1 negative, 1 positive). The negative control is prepared from a fixed cultured normal human male lymphoblast cell line (GM11854); the positive control is prepared from a fixed cultured human bladder carcinoma cell line (UM-UC-3). Store the control slides at –20°C in a sealed container with desiccant to protect them from humidity.
- Vysis FISH Pretreatment Reagent Kit (Part No. 32-801270/ List No. 02J03-032), which includes:
  - Vysis Protease (3×25 mg)
    Pepsin Activity 1:3000 to 1:3500
    NOTE: Pepsin digests not less than 3000 and not more than 3500 times its weight of coagulated egg albumin.
  - Vysis Pepsin Buffer (3×50 mL)
    10 mM HCl
  - Vysis PBS (2×250 mL)
    1X Phosphate Buffered Saline
  - Vysis 100X $MgCl_2$ (3×0.5 mL)
    2M $MgCl_2$
  - Vysis 20X SSC (66 g)
- 10% neutral buffered formalin
- Carnoy's Fixative (3:1 (v:v) methanol:glacial acetic acid). Prepare fresh daily.
- Immersion oil for appropriate microscope objectives. Store at room temperature (15 to 30°C).
- Ethanol (100%). Store at room temperature.
- Concentrated (12N) HCl
- 1N NaOH
- Purified water (Milli-Q). Store at room temperature.
- Rubber cement
- Ultra-pure, formamide.

Specimen Preservation

- Carbowax (2% polyethylene glycol in 50% ethanol). Suggested source: Sigma-Aldrich.
- ThinPrep PreservCyt Solution, Hologic (Cytyc) Corp.

Laboratory Equipment

- Glass coverslips (12 mm round and 18 mm square glass coverslips are recommended)
- 12-well, 6 mm circle microscope slides. Suggested type: Shandon
- Microliter pipettors (1 to 10 µL and 20 to 200 µL) and clean tips
- Conical centrifuge tubes (15 and 50 mL)
- Timer (±1 second)
- Magnetic stirrer
- Vortex mixer
- Microcentrifuge
- Bench-top centrifuge
- Graduated cylinder
- Water baths (37±1°C and 73±1°C)
- Humidified hybridization box
- Air incubator (37±1°C)
- Forceps
- Disposable syringe (5 mL)
- Coplin jars (10) Suggested type: Wheaton Product No. 900570
- Epi-fluorescence microscope equipped with a 100-watt mercury lamp and recommended filters (yellow single-bandpass, aqua single-bandpass, DAPI single-bandpass, and green/red dual-bandpass)
- Phase-contrast microscope equipped with a 20X objective
- pH meter and pH paper
- Calibrated thermometer
- 0.45 µm pore filtration unit
- Desiccant
- Automated Codenaturation Assay options:
  - HYBrite System—Refer to System Manual for user instructions.
  - ThermoBrite System—Refer to System Manual for user instructions.

- Automated Pretreatment Assay option:
  - VP 2000 Processor—Refer to System Manual for user instructions

Microscope Equipment and Accessories

Microscope: An epi-illumination fluorescence microscope is required for viewing the hybridization results. *If an existing fluorescence microscope is available, it should be checked to be sure that it is operating properly to ensure optimum viewing of fluorescence in situ hybridization assay specimens*. A microscope used with general DNA stains such as DAPI, Propidium Iodide, and quinacrine may not function adequately for FISH assays. Routine microscope cleaning and periodic preventive maintenance by the manufacturer's technical representative are recommended.

NOTE: Often, a presumed failure of reagents in an in situ assay may actually indicate that a malfunctioning or suboptimal fluorescence microscope or incorrect filter set is being used to view a successful hybridization assay.

Excitation Light Source: The excitation lamp is the source of the light that excites the fluorophores to fluoresce. Unless the excitation lamp is properly aligned, the optimum image will not be generated. A 100-watt mercury lamp with life maximum of about 200 hours is the recommended excitation source. Record the number of hours that the bulb has been used and replace the bulb before it exceeds the rated time.

Objectives: The objective has a profound influence on the brightness, resolution, and general quality of the image. Use oil immersion fluorescence objectives with numeric apertures ≥0.75 when using a microscope with a 100-watt mercury lamp. A 40X objective, in conjunction with 10X eyepieces, is suitable for scanning. For UroVysion analysis and signal enumeration, satisfactory results can be obtained with a 60X, 63X, or 100X oil immersion achromat-type objective.

Immersion Oil: The immersion oil used with oil immersion objectives should be one formulated for low autofluorescence and specifically for use in fluorescence microscopy.

Filters: Fluorescence microscope filter sets optimized for use with the CEP and LSI DNA probe kits are available from Abbott Molecular for most microscope models. Performance characteristics of the UroVysion assay with other filters must be determined and validated by the user. The recommended filter sets for the UroVysion Kit are the yellow single-bandpass, aqua single-bandpass, DAPI single-bandpass, and green/red dual-bandpass. Hybridization of the LSI 9p21 and CEP 3, 7, and 17 probes to their target regions is marked by gold, red, green, and aqua fluorescence, respectively. The remaining nuclear DNA will fluoresce blue with the DAPI stain.

Preparation of Working Reagent

1% Formaldehyde Solution

To prepare, add together:

12.5 mL	10% Neutral Buffered Formalin
37 mL	1X PBS
0.5 mL	100X $MgCl_2$ (1 tube from Vysis FISH Pretreatment Reagent Kit)
50 mL	Final volume

Mix thoroughly. Pour the solution into a Coplin jar. Discard used solution after using 1 week. Store unused solution at 2 to 8°C for up to 6 months.

20X SSC (3M sodium chloride, 0.3M sodium citrate, pH 5.3)

To prepare 20X SSC pH 5.3, add together:

66 g	20X SSC
200 mL	Purified water
250 mL	Final volume

Mix thoroughly. Measure pH at room temperature with a pH meter. Adjust pH to 5.3 with concentrated HCl. Bring the total volume to 250 mL with purified water. Filter through a 0.45 µm pore filtration unit. Store at room temperature for up to 6 months.

Denaturing Solution (70% Formamide/2X SSC pH 7.0 to 8.0)

NOTE: Not required for Automated (HYBrite or ThermoBrite) Codenaturation Assay.

To prepare denaturing solution, add together:

49 mL	Formamide
7 mL	20X SSC pH 5.3
14 mL	Purified water
70 mL	Final Volume

Mix thoroughly. Measure pH at room temperature using pH paper to verify that the pH is 7.0 to 8.0. This solution can be used for up to 1 week. Check pH prior to each use. Store at 2 to 8°C in a tightly capped container when not in use.

Ethanol Wash Solutions

Prepare v/v dilutions of 70% and 85% using 100% ethanol and purified water. Dilutions may be used for 1 week unless evaporation occurs or the solution becomes diluted due to excessive use. Store at room temperature in tightly capped containers when not in use.

0.4X SSC/0.3% NP-40

To prepare, add together:

20 mL	20X SSC pH 5.3
877 mL	Purified water
3 mL	NP-40
1000 mL	Final Volume

Mix thoroughly. Measure pH at room temperature using a pH meter. Adjust pH to 7.5±0.2 with 1N NaOH. Adjust volume to 1 liter with purified water. Filter through 0.45 µm pore filtration unit. Discard used solution at the end of each day. Store unused solution at room temperature for up to 6 months.

2X SSC/0.1% NP-40

To prepare, add together:

100 mL	20X SSC pH 5.3
849 mL	Purified water
1 mL	NP-40
1000 mL	Final Volume

Mix thoroughly. Measure pH at room temperature using a pH meter. Adjust pH to 7.0±0.2 with 1N NaOH. Adjust volume to 1 liter with purified water. Filter through 0.45 µm pore filtration unit. Discard used solution at the end of each day. Store unused solution at room temperature for up to 6 months.

WARNINGS AND PRECAUTIONS

IVD In Vitro Diagnostic Medical Device

1. For In Vitro Diagnostic Use
2. All biological specimens should be treated as if capable of transmitting infectious agents. The ProbeChek Control Slides recommended for use with this kit are manufactured from human cell lines that have been fixed in Carnoy's fixative. *Because it is often impossible to know which might be infectious, all human specimens and control slides should be treated with universal precautions. Guidelines for specimen handling are available from the U.S. Centers for Disease Control and Prevention*.[22]
3. Hybridization conditions may be adversely affected by the use of reagents other than those provided by Abbott Molecular.
4. Failure to follow all procedures for slide denaturation, hybridization, and detection may cause unacceptable or erroneous results.
5. Fluorophores are readily photobleached by exposure to light. To limit this degradation, handle all solutions containing fluorophores in reduced light. This includes all steps involved in handling the hybridized slide. Carry out all steps that do not require light for manipulation (incubation periods, slide drying, etc) in the dark.
6. UroVysion probe mixture contains formamide, a teratogen. Avoid contact with skin and mucous membranes. Refer to MSDS for more information.
7. Calibrated thermometers are required for measuring temperatures of solutions, water baths, and incubators.
8. All hazardous materials should be disposed of according to your institution's guidelines for hazardous disposal.

UroVysion DNA Probe Mixture

CAUTION: This preparation contains human sourced and/or potentially infectious components. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. These reagents and human specimens should be handled as if infectious using safe laboratory procedures, such as those outlined in Biosafety in Microbiological and Biomedical Laboratories,[24] OSHA Standards on Bloodborne Pathogens,[25] CLSI Document M29-A3,[26] and other appropriate biosafety practices.[27] Therefore all human sourced materials should be considered infectious.

These precautions include, but are not limited to, the following:

- Wear gloves when handling specimens or reagents.
- Do not pipette by mouth.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in areas where these materials are handled.
- Clean and disinfect spills of specimens by including the use of a tuberculocidal disinfectant such as 1.0% sodium hypochlorite or other suitable disinfectant.[24]
- Decontaminate and dispose of all potentially infectious materials in accordance with local, state, and federal regulations.[27]

UroVysion DNA Probe Mixture

Danger

Hazard-determining components of labeling: Formamide

H360D	May damage the unborn child.
P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P281	Use personal protective equipment as required.
P308+P313	IF exposed or concerned: Get medical advice/attention.
P405	Store locked up.
P501	This material and its container must be disposed of in a safe way.

NP-40

Danger

Hazard-determining components of labeling: Triton X-100

H302	Harmful if swallowed.
H318	Causes serious eye damage.
H412	Harmful to aquatic life with long lasting effects.
P280	Wear protective gloves/protective cloting/ eye protection.
P264	Wash hands thoroughly after handling.
P273	Avoid release to the environment.
P305+ P351+ P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P301+P312	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P501	This material and its container must be disposed of in a safe way.

Safety Data Sheet Statement: Important information regarding the safe handling, transport, and disposal of this product is contained in the Safety Data Sheet.

NOTE: Safety Data Sheets (SDS) for all reagents provided in the kits are available upon request from the Abbott Molecular Technical Services Department.

SPECIMEN COLLECTION AND TRANSPORT

The UroVysion Kit is designed for use on voided urine specimens. Perform urine collection (≥ 33 mL) at the physician's office. Mix voided urine 2:1 (v:v) with preservative; Carbowax (2% polyethylene glycol in 50% ethanol) or PreservCyt preservatives are recommended. Transfer to a 50 mL centrifuge tube(s) or other tightly-capped plastic container. Use of any other preservative must be validated by the individual laboratory. If urine is not shipped immediately after collection, refrigerate immediately and ship via overnight courier within 24 hours.

The preferred storage and shipping conditions are on ice packs, but specimens may be stored and shipped at temperatures up to 25°C. Urine stored in Carbowax or PreservCyt under these conditions has been shown to be stable for 1 week; however, it is recommended that specimens be processed to the point of fixed cell pellets (see Sample Processing, step 7) within 72 hours of collection. Performance characteristics of the UroVysion test under any other conditions must be determined and validated by the user.

SPECIMEN PROCESSING AND PREPARATION

Sample Processing

1. Centrifuge urine in a 50 mL centrifuge tube at 600*g* for 10 minutes at room temperature (15 to 30°C).
2. Remove the supernatant to within approximately 1 to 2 mL of the cell pellet, being careful not to disturb the pellet.
3. Resuspend the pellet in the remaining 1 to 2 mL of supernatant and transfer the contents to a 15 mL conical centrifuge tube. Rinse the 50 mL tube with 10 mL of 1X PBS and transfer the contents to the 15 mL tube.
   NOTE: Pellets from the same patient specimen may be combined.
4. Centrifuge sample(s) at 600*g* for 10 minutes at room temperature.
5. Remove the supernatant to within approximately 0.5 mL of the cell pellet.
6. Resuspend pellet in the remaining 0.5 mL of supernatant. Slowly add 1 to 5 mL of fresh fixative (3:1, methanol:acetic acid), dropwise at first, with frequent agitation.
7. Let fixed specimens stand at −20°C for a minimum of 30 minutes.
   NOTE: Specimens may be stored overnight or longer (up to 10 days) at this step.
8. Centrifuge sample(s) at 600*g* for 5 minutes at room temperature. Carefully remove the supernatant.
   NOTE: If pellet is not visible or barely visible, further washing of the pellet is not recommended in order to avoid cell loss. Instead, proceed to step 11. If sample has been stored overnight or longer, resuspend in fresh fixative prior to slide preparation.
9. Wash pellet by resuspending in 1 to 5 mL fixative.
10. Centrifuge sample(s) at 600*g* for 5 minutes at room temperature. Repeat steps 8 and 9 twice.
11. After centrifugation of cell suspension in fixative:
    - If cell pellet is very small and hardly visible, CAREFULLY remove as much fixative as possible, leaving approximately 100 µL solution.
    - If cell pellet is easily visible, remove as much fixative as possible and add 0.5 to 1 mL fresh fixative to the cell pellet.
12. Proceed immediately with the slide preparation procedure.

Slide Preparation

Use 12-well slides.

1. Resuspend the cell pellet and apply 3 µL, 10 µL, and 30 µL of cell suspension on 3 slide circles (circle No. 1, 2, and 3).
2. Allow samples to air-dry.
3. Examine slide under a Phase-contrast microscope using a 20X objective.
4. Select the hybridization area (circle No. 1, 2, or 3) in which ~100 to 200 cells are visible in the field. The circle which best corresponds to the recommended cell density (ie, 100 to 200 cells per field) should be used for UroVysion hybridization.
   - If cell density is too low, even in circle No. 3, apply another 30 µL of cell suspension on circle No. 3. Allow sample to dry and examine under Phase-contrast microscope, repeat if necessary.
   - If cell density is too high, even in circle No. 1, dilute the cell suspension sample with fixative and repeat steps 1 through 4.

   NOTE: If an excessive amount of debris is present, follow pretreatment procedure and then select hybridization area.
5. Prepare at least 1 additional back up slide following slide preparation steps 1 through 4 above. Store additional slide(s) at −20°C in a box with desiccant.
   NOTE: Fixed slides are stable at −20°C for up to 12 months. Storing any remaining cell suspension at −20°C for up to 1 month in the event preparation of additional slides is necessary.

Slide Pretreatment

Slides must be pretreated and fixed prior to assay with the UroVysion Kit. The package insert for the FISH Pretreatment Reagent Kit (Part No. 32-801270/List No. 02J03-032) contains detailed instructions.

Manual Pretreatment Assay	Optional Automated Pretreatment Assay
• Allow slide(s) to completely dry at room temperature.	• Allow slide(s) to completely dry at room temperature.
• Immerse slide(s) in 2X SSC for 2 minutes (2 to 2.5 min) at 73±1˚C.	• Immerse slide(s) in 2X SSC for 2 minutes (2 to 2.5 min) at 73±1˚C.
• Immerse slide(s) in protease solution for 10 minutes (±1 min) at 37±1˚C.	• Immerse slide(s) in protease solution for 10 minutes (±1 min) at 37±1˚C.
• Wash slide(s) in 1X PBS for 5 minutes (±1 min) at room temperature.	• Wash slide(s) in 1X PBS for 5 minutes (±1 min) at room temperature.
• Fix slides in 1% formaldehyde for 5 minutes (±1 min) at room temperature.	• Fix slides in 1% formaldehyde for 5 minutes (±1 min) at room temperature.
• Wash slides in 1X PBS for 5 minutes (±1 min) at room temperature.	• Wash slides in 1X PBS for 5 minutes (±1 min) at room temperature.
• Dehydrate slide(s) by immersing in 70% ethanol solution at room temperature. Allow the slide(s) to stand in the ethanol wash for at least 1 minute. Repeat with 85% ethanol, followed by 100% ethanol.	• Dehydrate slide(s) by immersing in 70% ethanol solution at room temperature. Allow the slide(s) to stand in the ethanol wash for at least 1 minute. Repeat with 85% ethanol, followed by 100% ethanol.
• Allow slides to dry completely.	• Dry slides at 25˚C (air-drying station) for 3 minutes or until completely dry.
• Proceed with the UroVysion assay protocol.	• Proceed with the UroVysion assay protocol.

FISH PROCEDURE

UroVysion Assay

Manual Assay: (For optional Automated [HYBrite or ThermoBrite] Codenaturation Assay, see below)

NOTE: The timing for preparing the probe solution (see Probe Preparation, steps 1 to 3) should be carefully coordinated with denaturing the specimen DNA (steps 1 to 7) so that both will be ready for the hybridization step at the same time.

Denaturation of Specimen DNA:

1. Prewarm the humidified hybridization chamber (an airtight container with a piece of damp blotting paper or paper towel approximately 1 in × 3 in taped to the side of the container) to 37±1˚C by placing it in the 37±1˚C incubator prior to slide preparation. Moisten the blotting paper or paper towel with water before each use of the hybridization chamber.
2. Add denaturing solution to Coplin jar and place in a 73±1˚C water bath for at least 30 minutes, or until the solution temperature reaches 73±1˚C. Verify the solution temperature before use.
   NOTE: If solution has been stored at 2 to 8˚C, allow solution and Coplin jar to reach room temperature before placing in water bath.
3. Denature the specimen DNA by immersing the prepared slides in the denaturing solution at 73±1˚C (4 slides per jar) for 5 minutes (±1 min). Do not denature more than 4 slides at 1 time per Coplin jar; if denaturing fewer than 4 slides, supplement with blank glass slides.
   NOTE: Verify the solution temperature inside the Coplin jar before each use.
4. Using forceps remove the slide(s) from the denaturing solution and immediately place into a 70% ethanol wash solution at room temperature. Agitate the slide to remove the formamide. Allow the slide(s) to stand in the ethanol wash for at least 1 minute.
5. Remove the slide(s) from 70% ethanol. Repeat step 4 with 85% ethanol, followed by 100% ethanol.
6. Drain the excess ethanol from the slide by touching the bottom edge of the slide to a blotter, and wipe the underside of the slide dry with a laboratory wipe.
7. Dry the slide(s) on a 45 to 50˚C slide warmer for up to 2 minutes.

Probe Preparation

1. Remove the UroVysion probe from −20˚C storage and allow to warm to room temperature. Vortex to mix. Spin the tubes briefly (1 to 3 seconds) in a microcentrifuge to bring the contents to the bottom of the tube. Gently vortex again to mix.
2. Heat UroVysion probe solution for 5 minutes in the 73±1˚C water bath.
3. Place probe solution on a 45 to 50˚C slide warmer.

Hybridization

1. Apply 3 µL of probe solution to the selected target area of slide. Immediately, place a 12 mm round glass coverslip over the probe. Carefully apply light pressure to the coverslip to allow the probe solution to spread evenly under the coverslip. Air bubbles will interfere with hybridization and should be avoided. The remaining probe solution should be returned to −20˚C storage immediately after use.
2. Seal coverslip with rubber cement as follows: Draw the rubber cement into a 5 mL syringe. Eject a small amount of rubber cement around the periphery of the coverslip overlapping the coverslip and the slide, forming a seal around the coverslip.
3. Place slides in the prewarmed humidified hybridization chamber. Cover the chamber with a tight lid and incubate at 37±1˚C overnight (approximately 16 hours).
4. Proceed to Post-Hybridization Washes.

Optional Automated (HYBrite or ThermoBrite) Codenaturation Assay:

Probe Preparation and Application

1. Remove the UroVysion probe from −20˚C storage and allow to warm to room temperature (15 to 30˚C). Vortex to mix. Spin the tube briefly (1 to 3 seconds) in microcentrifuge to bring the contents to the bottom of the tube. Gently vortex again to mix.
2. Apply 3 µL of probe solution to the selected target area of slide. Immediately, place a 12 mm round glass coverslip over the probe. Carefully apply light pressure to the coverslip to allow the probe solution to spread evenly under the coverslip. Air bubbles will interfere with hybridization and should be avoided. The remaining probe solution should be returned to −20˚C storage immediately after use.
3. Seal coverslip with rubber cement as follows: Draw the rubber cement into a 5 mL syringe. Eject a small amount of rubber cement around the periphery of the coverslip overlapping the coverslip and the slide, forming a seal.

Denaturation of Specimen DNA and Hybridization on the HYBrite System:

1. Moisten a paper towel with water and place the towel in the channels along the heating surface.
2. Turn the HYBrite instrument on.
3. Set the program for Melt Temp 73˚C and Melt Time 2 minutes (denaturation), and Hybridization Temperature 39˚C and Hybridization Time 4 to 16 hours.
4. When prompted, place slides on heating surface of the instrument. Supplement with blank glass slides, as necessary. Ensure that the slides lay flat on the heating surface.
5. Close HYBrite lid and run program.

Denaturation of Specimen DNA and Hybridization on the ThermoBrite System:

1. Insert 2 humidity cards into the slot positions of the unit lid. Moisten each card with 8 to 10 mL of distilled or deionized water. *Refer to ThermoBrite Operator's Manual for reuse of humidity cards in subsequent runs.*
2. Turn the ThermoBrite unit on.
3. Set the program for Denat Temp 76˚C and Denat Time 3 minutes (denaturation) and Hyb Temp 39˚C and Hyb Time 14 to 18 hours (hybridization).
4. When prompted, place slides on heating surface of the instrument. Ensure the slides lay flat and rest into the marked positions in the slide locator.
5. Close ThermoBrite lid and run program.

Post-Hybridization Washes *(Manual and Automated assays)*

1. Thirty minutes prior to washing, fill a Coplin jar with 0.4X SSC/0.3% NP-40 and place in a 73±1˚C water bath. Using a calibrated thermometer, check the temperature of the solution inside the jar before adding slides for the wash procedure. The solution temperature should be 73±1˚C.
2. Fill a second jar with 2X SSC/ 0.1% NP-40 and place at room temperature. Discard both wash solutions after 1 day of use.
3. Remove the rubber cement and coverslip from the slide(s).
   NOTE: Remove rubber cement and coverslip from 1 slide at a time and place immediately into the 0.4X SSC/0.3% NP-40 Coplin jar.
4. Place slide(s) in the 0.4X SSC/0.3% NP-40 immediately after removing the coverslip. When all the slides are in the jar (maximum of 4) incubate for 2 minutes at 73±1˚C. Do not wash more than 4 slides at a time in the same jar; supplement with blank glass slides if necessary.

NOTE: Placing an individual slide in the jar should not require more than a few seconds; if it does, then be sure that no slide is in the wash buffer for more than 2 minutes. After removal of the slides, allow the temperature to return to 73 ± 1°C before washing more slides.

5. After 2 minutes remove the slide(s) from the wash solution and place the slide(s) in the Coplin jar containing 2X SSC/0.1% NP-40 at room temperature. Incubate for 5 seconds to 1 minute.
6. Remove the slide(s) from the wash solution and place vertically in a dark area (such as a drawer) on a paper towel to dry completely.
7. Apply 10 µL of DAPI II onto the target area and place a coverslip (18 mm square is recommended) over the DAPI II solution, avoiding air bubbles. Store the slide(s) in the dark prior to signal enumeration.

Slide Storage

Store hybridized slides (with coverslips) at – 20°C in the dark. After removing from – 20°C storage, allow slide(s) to reach room temperature prior to viewing using fluorescence microscopy.

Interpretation of Results

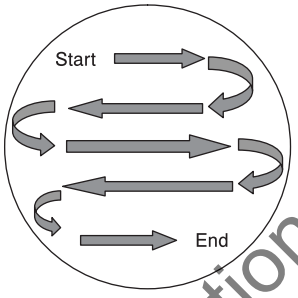
UroVysion probe signals and DAPI counterstain should be viewed with the following filters:

- DAPI single-bandpass
- Aqua single-bandpass (chromosome 17)
- Yellow (Gold) single-bandpass (9p21 locus)
- Red/Green dual-bandpass (chromosomes 3 and 7)

An epi-fluorescence microscope equipped with a 100-watt mercury lamp is strongly recommended. The DAPI counterstain will cause the nucleus to fluoresce bright blue.

Analysis of Specimen Slides

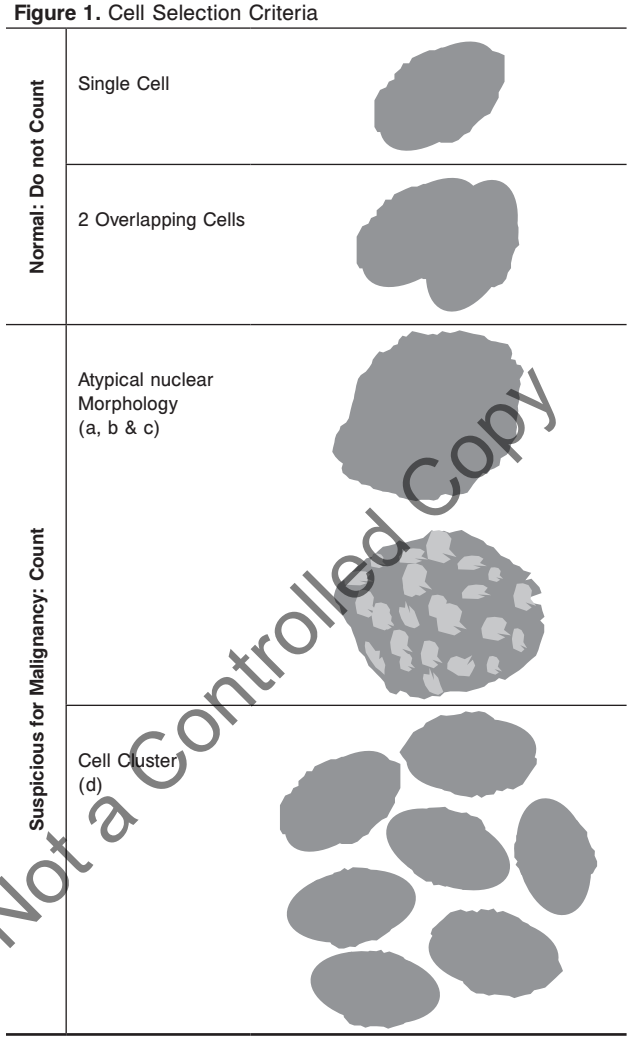
1. Use the prescribed filters (see above) and a magnification of 400X for scanning (600X to 1000X for analysis, see step 5 below).
2. Adjust the depth of focus and become familiar with the size and shape of the target signals and noise (debris).
3. Begin analysis in the upper left quadrant of the target area. Scan fields from left to right and top to bottom, without rescanning the same areas (see diagram below).
   NOTE: There are approximately 70 to 80 fields of view per slide.



4. Use the following criteria (see **Figure 1**) to select cells suspicious for malignancy (morphologically abnormal):
   a. large nuclear size
   b. irregular nuclear shape
   c. "patchy" DAPI staining
   d. cell clusters (do not count overlapping cells in clusters)

   NOTE: Begin with those cells which appear morphologically abnormal. If few morphologically abnormal cells are present, select the largest cells, or those with the largest nuclei. If morphologically abnormal cells are not readily apparent, the entire sample should be scanned and nuclei representing the <u>most</u> morphologically abnormal cells should be scored first.

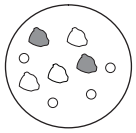
Figure 1. Cell Selection Criteria



5. Increase magnification to 600X to 1000X. Focus up and down to find all of the signals present in the nucleus.
6. Determine the number of signals for all 4 probes in 25 morphologically abnormal cells*# using the filters listed above (see **Figures 2, 3** & **4**).
7. Record the chromosome pattern only if:
   - there is a gain (ie, 3 or more signals) of 2 or more of chromosomes 3 (red), 7 (green), or 17 (aqua), **<u>or</u>**
   - there is a loss of both copies of LSI 9p21.

   If chromosomes 3, 7, or 17 show the loss of both chromosomes, consider the cell to be uninterpretable due to hybridization failure.

   NOTE: If surrounding cells show abnormal chromosome patterns, as described above, these cells should be recorded, even if they are not morphologically abnormal.



For Illustration only, not to scale.

Morphologically "normal" cell. **Do not score.** See note in step 7 above for exception.

Morphologically abnormal cell with diploid chromosome pattern. **Count in total number of cells analyzed, but do not record chromosome pattern.**

Morphologically abnormal cell with abnormal chromosome pattern. **Record the chromosome pattern.**

*# If morphologically abnormal cells are not readily apparent, the entire sample should be scanned and nuclei representing the <u>most</u> morphologically abnormal cells should be scored first.

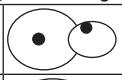
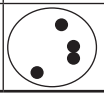
8. Record the total number of morphologically abnormal cells viewed (diploid and abnormal).
   NOTE: Though the individual signal counts are not recorded, cells with nondiploid counts having at least 1 signal for each of the 4 probes but not fitting the criteria specified in step 7 should be included, along with the diploid cells, in the overall total number of morphologically abnormal cells viewed.
9. If, after 25 morphologically abnormal cells have been analyzed,*# any of the following criteria have been met, **STOP** 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 **either**:
   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.

*# If morphologically abnormal cells are not readily apparent, the entire sample should be scanned and nuclei representing the most morphologically abnormal cells should be scored first.

Analysis of Quality Control Slides

For enumeration of quality control slides, follow steps 1 through 6 above. Enumerate **25 consecutive cells** and record the results. **Do not** select for morphologically abnormal cells only, or stop enumeration after detecting 4 or 12 cells as described above.

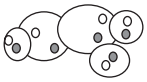
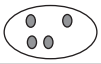
Figure 2. Single-Color Signal Counting Guide

1		Don't count, skip over. This could be 2 cells with 1 signal each or 1 twisted nucleus.
2		Count as 2 signals: 1 is very compact, the other is diffuse.*
3		Don't count, skip over. Observer cannot determine which cell contains the signals.
4		Count as 2 signals. One signal is split.*
5		Count as 3 signals.
6		Count as 4 signals.
7		Count as 3 signals. One is split.

* Count a diffuse signal as 1 signal if diffusion of the signal is contiguous and within an acceptable boundary; 2 signals connected by a visible link are considered a split signal and should be counted as 1 signal. A split or diffuse signal may occur as a result of variable DNA condensation within a nucleus, the extent of which is dependent upon the current stage of cell division; it does not indicate an additional copy of the chromosome in that cell.

Figure 3. Dual-Color Signal Counting Guide

Key: ○ = green probe
● = red probe

1		Don't count—nuclei are overlapping and all areas of both nuclei are not visible.
2		Count as 1 red signal and 1 green signal. The red signal is diffuse.*
3		Don't count. Nuclei are too close together to determine boundaries.
4		Count as 1 red signal and 1 green signal. The red signal is split.*
5		Count as 1 red signal and 2 green signals. One green signal is split and the red signal is split.*
6		Count as 2 red signals and 1 green signal.
7		Count as 3 red signals and 1 green signal.
8		Count as 4 red signals.

* Count a diffuse signal as 1 signal if diffusion of the signal is contiguous and within an acceptable boundary; 2 signals connected by a visible link are considered a split signal and should be counted as 1 signal. A split or diffuse signal may occur as a result of variable DNA condensation within a nucleus, the extent of which is dependent upon the current stage of cell division; it does not indicate an additional copy of the chromosome in that cell.

Figure 4. Examples of Chromosomally Normal and Abnormal Cells

Key: ○ = CEP 3 (red) ● = CEP 17 (aqua)
⊗ = CEP 7 (green) ● = LSI 9p21 (gold)

1		Chromosomally normal cell
2		Chromosomally abnormal—gains of CEP 3 and CEP 17
3		Chromosomally abnormal—homozygous loss of LSI 9p21

QUALITY CONTROL

Control slides must be run concurrently with patient slides to monitor assay performance and to assess the accuracy of signal viewing. One control slide (1 positive and 1 negative target per slide) must be processed for each specimen processing run, and with each new kit lot. Control slides must be hybridized with the UroVysion probe mixture along with study specimen slides.

Perform signal enumeration according to the instructions in the analysis of quality control slides section above. The signal enumeration results should be within the specifications on the data sheets provided with the control slides for acceptable test performance.

If control slides fail to meet the slide acceptance criteria, the assay may not have been performed properly or the UroVysion assay reagents may have performed inadequately. In no case should UroVysion test results be reported if assay controls fail. If control slides meet the acceptance criteria but the results are outside the specified range, the enumeration may not have been performed correctly and an independent, repeat analysis of the same slide may be appropriate. In the event of hybridization failure, with either the study specimen or the control slide(s), consult the troubleshooting guide in **Table 1**.

For clinical specimens, when interpretation of the hybridization signal is difficult the test is uninformative. If there are insufficient cells for analysis, the test is uninformative.

Patient specimens should be controlled according to standard laboratory procedure requirements. Hybridization quality and enumeration should be documented on an appropriate form. Hybridization quality and efficiency should be considered when evaluating results.

Table 1. Troubleshooting Guide

Problem	Probable Cause	Solution
• No signal or weak signals	• Inappropriate filter set used to view slides	• Use recommended filters
	• Microscope not functioning properly	• Call microscope manufacturer's technical representative
	• Improper lamps (ie, Xenon or Tungsten)	• Use a mercury lamp (100-watt recommended)
	• Mercury lamp too old	• Replace with a new lamp
	• Mercury lamp misaligned	• Realign lamp
	• Dirty and/or cracked collector lenses	• Clean and replace lens
	• Dirty or broken mirror in lamp house	• Clean or replace mirror
	• Hybridization conditions inappropriate	• Check denaturation and hybridization temperatures.
	• Inappropriate posthybridization wash temperature	• Increase hybridization time to at least 16 hours • Check temperature of 73±1°C water bath
	• Air bubbles trapped under coverslip and prevented probe access	• Apply coverslip by first touching the surface of the hybridization mixture
	• Inadequate protease digestion	• Check temperature of 37±1°C bath • Check that pH of buffer is 2.0±0.2 • Increase digestion time, up to 20 min
	• DNA loss (poor DAPI staining)	• Check fixation conditions
	• Probes improperly stored	• Store probes at −20°C in darkness
• Low signal specificity	• Hybridization conditions inappropriate	• Check denaturation and hybridization temperatures
	• Wash temperature too low	• Maintain wash temperature at 73±1°C
• Noisy background	• Inadequate wash stringency	• Check pH of wash buffers • Check temperature of 73±1°C bath • Provide gentle agitation during wash
• Excessively bright signal	• Probe concentration too high for your microscope	• Try to block some of the signal by placing a neutral density filter in the excitation pathway
• Cells structure not intact	• Sample was overdigested	• Reduce protease digestion time

INTERPRETATION OF RESULTS

A minimum of 25 morphologically abnormal cells are analyzed. The signal distribution for morphologically abnormal cells showing either a gain of multiple chromosomes (ie, 3 or more signals) for more than 1 of the following (CEP 3 red, CEP 7 green, or CEP 17 aqua) probes or a homozygous loss of 9p21 (ie, no signals for LSI 9p21 yellow) is recorded. Analysis continues until either ≥4 cells with gains of multiple chromosomes or ≥12 cells with homozygous loss of 9p21 are detected, or until the entire sample is analyzed. The total number of chromosomally abnormal cells, ie, cells with gains of multiple chromosomes or homozygous loss of 9p21, are determined; results are reported as positive or negative. Our clinical study found that specimens from patients positive for bladder cancer recurrence showed ≥4 cells with multiple chromosomal gains or ≥12 cells with loss of both copies of 9p21.

Results at or near the cutoff point (4 cells with gains of multiple chromosomes or 12 cells with homozygous loss of 9p21) should be interpreted with caution. The specimen slide should be re-enumerated by another technician to verify the results. If still in doubt, the assay should be repeated with a fresh specimen slide. If the test results are not consistent with the clinical findings, a consultation between the pathologist and the treating physician is warranted.

Reasons to Repeat the Assay

The following are situations requiring repeat assays with fresh specimen or existing slides and the appropriate control slides. Consult the troubleshooting guide (**Table 1**) for probable causes and the actions needed to correct specific problems.

1. If 1 or both of the control slide targets fail to meet the slide acceptance criteria, the specimen slide results are not reliable, and the assay must be repeated.
2. If there are fewer than 25 evaluable nuclei, the test is uninformative and the assay should be repeated.
3. If, upon assessing the slide quality, any of the technical aspects (signal intensity, background, or cross-hybridization) are unsatisfactory, the assay must be repeated.

LIMITATIONS

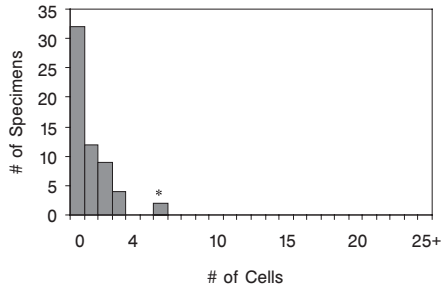
1. The UroVysion Kit has been optimized for identifying and quantitating chromosomes 3, 7, and 17, and locus 9p21 in human urine specimens.
2. The performance of the UroVysion Kit was validated using the procedures provided in this package insert only. Modifications to these procedures may alter the 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, eg, 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, eg, ureter, urethra, renal, and/or prostate in males, and further patient follow-up is justified.
   In a study conducted on patients with hematuria (see Symptomatic Patients: Performance vs Standard of Care for details on this clinical study) 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 (eg, cytology, cystoscopy) are positive, the standard procedures take precedence over the UroVysion test. Although the UroVysion 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 5 mm could not be detected by UroVysion FISH.[23] UroVysion FISH results are dependent on the amount of tumor cells that are deposited on the slide.

EXPECTED VALUES

Values Among Healthy Subjects

FISH analysis with the UroVysion Kit was performed with urine specimens from 59 healthy donors (50 nonsmokers and 9 smokers), as part of an assay specificity study (see also Specificity section below). All 59 healthy donor specimens were negative by UroVysion. The distribution of chromosomally abnormal cells in this population is shown in **Figure 5**. Note that there were 2 specimens with ≥4 abnormal cells (identified by * in **Figure 5**), however in both cases all 6 abnormal cells showed homozygous loss of 9p21 only. The cutoff for 9p21 loss is ≥12 cells, thus these 2 specimens are considered negative.

Figure 5. Distribution of Chromosomally Abnormal Cells Among Healthy Subjects



Values Among Patients with History of Bladder Cancer

In a prospective, longitudinal study of patients with a history of bladder cancer, 62 patients experienced a recurrence within 17 months as determined by cystoscopy/histology (see Clinical Studies; Bladder Cancer Recurrence: Performance vs. Standard of Care section for details regarding this clinical study). The distribution of chromosomally abnormal cells among these 62 patients is shown in **Figure 6**. The distribution of chromosomally abnormal cells among the 114 patients who did not experience a recurrence based on standard clinical measures (cystoscopy/histology) is shown in **Figure 7**.

Figure 6. Distribution of Chromosomally Abnormal Cells Among Patients Experiencing a Recurrence of Bladder Cancer as Determined by Cystoscopy/Histology

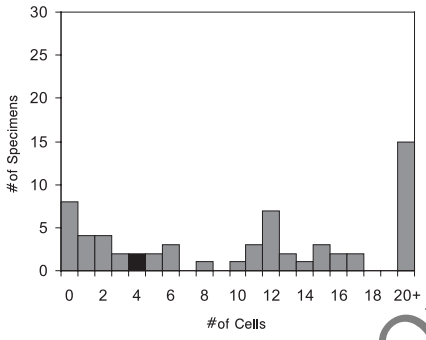
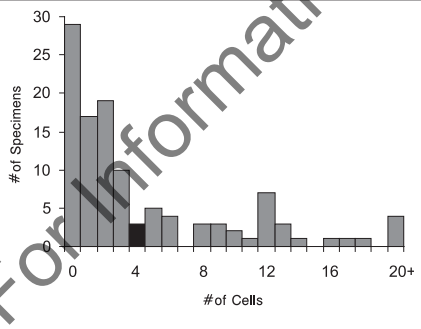


Figure 7. Distribution of Chromosomally Abnormal Cells Among Patients Negative for Recurrence of Bladder Cancer as Determined by Cystoscopy/Histology



Values Among Patients with Hematuria but No History of Bladder Cancer

In a prospective, longitudinal study of patients symptomatic for bladder cancer, 50 patients were diagnosed with bladder cancer, as determined by cystoscopy/histology, and 1 patient was diagnosed with ureteral cancer (see Clinical Studies; Symptomatic Patients: Performance vs. Standard of Care section for details regarding this clinical study). The distribution of chromosomally abnormal cells among these 51 patients is shown in **Figure 8**. The distribution of chromosomally abnormal cells among the 419 patients who did not have bladder cancer, based on standard clinical measures (cystoscopy/histology), is shown in **Figure 9**.

Figure 8. Distribution of Chromosomally Abnormal Cells Among Symptomatic Patients Positive for Bladder Cancer as Determined by Cystoscopy/Histology

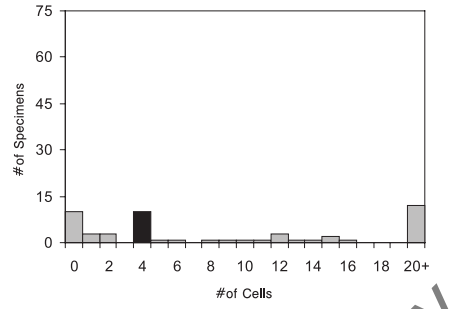
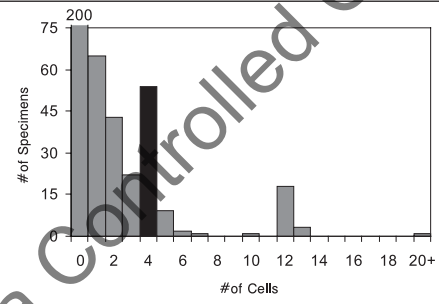


Figure 9. Distribution of Chromosomally Abnormal Cells Among Symptomatic Patients Negative for Bladder Cancer as Determined by Cystoscopy/Histology



PERFORMANCE CHARACTERISTICS

Non-Clinical

Hybridization Efficiency/Informative vs Non-Informative Results

On the ProbeChek quality control slides run in conjunction with the clinical trials, 1.2% (4/328) (95% CI: 0.3%, 3.1%) of the targets failed due to lack of hybridization. These slides are prepared from cultured human bladder carcinoma (positive target) and normal lymphoblast (negative target) cell lines, and represent the best-case scenario for hybridization efficiency. Thus, under these conditions, the hybridization efficiency was found to be 98.8% (324/328) (95% CI: 96.9%, 99.7%), with < 2% cells having no signal for any of the probes. On the subset of 6 control slides assayed using the automated pretreatment (VP 2000 Processor) and automated UroVysion assay (HYBrite) procedures, the hybridization efficiency was 100% (6/6) (95% CI: 54.1%, 100%).

In a reproducibility study conducted using the manual pretreatment and manual UroVysion assay procedures on specimens prepared from human bladder carcinoma cell lines, 76 of 80 specimens yielded informative results on the first attempt. Of the 4 uninformative specimens, 3 were due to lack of hybridization. Therefore the hybridization efficiency was found to be 96.2% (76/79) (95% CI: 89.3%, 99.2%), based on the following definition:

% Hybridization Efficiency =
100-[hybridization failures/(informative results + hybridization failures)] × 100

In a specificity study conducted using the manual pretreatment and manual UroVysion assay procedures on urine specimens from patients with no history of bladder cancer, 230 of 309 specimens yielded informative results on the first attempt and 18 of the uninformative results were due to lack of hybridization, resulting in a hybridization efficiency of 93% (230/248) (95% CI: 88.8%, 95.6%), based on the definition above. The remaining non-informative assays were the result of poor specimen quality (eg, insufficient number of cells) or technical error (eg, oil under coverslip).

Repeat assays were conducted on 67 specimens; 12 of the 79 specimens with non-informative initial results had insufficient volume remaining to repeat the assay. Of the 67 repeat assays, 45 yielded informative results, leaving 34 specimens classified as “non-informative” (including the 12 cases with insufficient volume for repeat assay). In summary, 89% (275/309) (95% CI: 85.0%, 92.3%) of the cases yielded an informative result on the first or second attempt.

Similarly, in a clinical study conducted using the manual pretreatment and manual UroVysion assay procedures on urine specimens from patients with a history of bladder cancer, 175 of 251 specimens yielded informative results on the first attempt, and 26 of the 76 uninformative results were due to lack of hybridization. The hybridization efficiency among these specimens was found to be 87.1% (175/201) (95% CI: 81.6%, 91.4%), based on the definition above. The remaining non-informative assays were the result of poor specimen quality (eg, insufficient number of cells) or technical error (eg, broken slide).

Repeat assays were conducted manually on 70 specimens; 6 of the 76 specimens had insufficient volume remaining to repeat the assay. Of the 70 repeat assays, 59 yielded informative results, leaving 17 specimens classified as "non-informative" (including the 6 cases with insufficient volume for repeat assay). In summary, 93.2% (234/251) (95% CI: 89.4%, 96.0%) of the cases yielded an informative result on the first or second attempt.

In a clinical study conducted using the automated UroVysion assay procedure on urine specimens from patients symptomatic for bladder cancer, 521 of 570 specimens (497 eligible patients plus 73 follow-up visits) yielded informative results on the first attempt and 5 of the 49 uninformative results were due to lack of hybridization. The hybridization efficiency among these specimens was found to be 99.0% (521/526) (95% CI: 97.8%, 99.7%), based on the definition above. The remaining non-informative assays were the result of poor specimen quality (eg, insufficient number of cells) or technical error (eg, broken slide or QC [Quality Control] slide failure). On the subset of 44 specimens for which the automated pretreatment procedure was also used, the hybridization efficiency was 96.7% (29/30) (95% CI: 82.8%, 99.9%).

Repeat assays were conducted on 43 specimens; 6 of the 49 specimens had insufficient volume remaining to repeat the assay. Of the 43 repeat assays, 26 yielded informative results, leaving 23 specimens classified as "non-informative" (including the 6 cases with insufficient volume for repeat assay). In summary, 96.0% (547/570) (95% CI: 94.0%, 97.0%) of the cases yielded an informative result on the first or second attempt.

To summarize, under all of these conditions, which simulate the normal clinical practice, the hybridization efficiency was found to be ≥87%. The studies showed also that hybridization efficiencies between specimens processed using the manual versus automated procedures were equivalent.

Analytical Specificity

Locus specificity studies were performed with metaphase spreads according to standard Abbott Molecular QC protocols. A total of 42 metaphase spreads were examined sequentially by reverse DAPI banding to identify chromosomes 3, 7, and 17, and the 9p21 locus, followed by UroVysion. No cross-hybridization to other chromosome loci was observed in any of the 42 cells examined; hybridization was limited to the intended target regions of the 4 probes.

Interference

Three voided urine pools (1 male, 1 female, 1 male/female mix) from normal healthy volunteers were spiked with the substances listed in **Table 2** and assayed with the UroVysion kit to test for possible assay interference. Replicate samples for each urine pool were evaluated for each substance (ie, 6 samples per substance tested); 25 consecutive cells were enumerated for each specimen. No interference was detected from any of the substances tested; results from all samples were negative (ie, <4 abnormal cells as defined in this package insert). The highest concentrations tested for each substance are shown in **Table 2**. Note that conducting this study on urine specimens from bladder cancer patients was not feasible due to the volume necessary to obtain enough cells to replicate the specimen between conditions. Hence the assay interference on specimens containing morphologically abnormal cells was not assessed.

Table 2. Substances Tested for Assay Interference

Substance	Highest Concentration Tested
Possible Urine Constituents	
Albumin	1.0 g/dL
Ascorbic Acid	5 g/dL
Bilirubin (unconjugated)	2 mg/mL
Hemoglobin	100 mg/mL
IgG	10 mg/dL
Red Blood Cells (human)	1×10^6 cells/mL
White Blood Cells (human)	1×10^6 cells/mL
Sodium Chloride	730 mg/dL
Uric Acid	250 mg/dL
Caffeine	117 mg/dL
Ethanol	1% (v/v)
Nicotine	28 mg/dL
Possible Microbial Contaminants	
Candida albicans	2.5×10^{10} CFU/mL
Escherichia coli	2.5×10^{10} CFU/mL
Pseudomonas aerugenosa	2.5×10^{12} CFU/mL
Therapeutic Agents	
Acetaminophen	5.2 g/dL
Acetylsalicylic Acid	5.2 g/dL
Ampicillin	600 mg/dL
BCG	20 mg/dL
Doxorubicin-HCl	10 mg/dL
Mitomycin C	10 mg/dL
Nitrofurantoin	50 mg/dL
Phenazopyridine-HCl	200 mg/dL
Thiotepa	10 mg/dL
Trimethoprin	50 mg/dL
Preservatives	
Carbowax	2% Carbowax/50% ethanol solution (33 mL urine with 17 mL preservative)
UroCor, Inc. fixative	50/50 with urine
CytoRichRed (Autocyte)	50/50 with urine
Saccamono's solution	50/50 with urine
PreservCyt solution (Cytyc)	50/50 with urine
100% Ethanol	50/50 with urine

Reproducibility

Reproducibility of Patient Samples

Conducting reproducibility studies on urine specimens from bladder cancer patients was not feasible; this is because 1 patient cell pellet does not yield enough cells to replicate the specimen between observers. Hence the reproducibility of results on morphologically abnormal cells were not assessed. Absent a comparison of replicate measures, the magnitude of results variation introduced by specimen manipulation, staining and counting errors is unknown. The statistics for small numbers of events imply a substantial coefficient of variation for samples with abnormal cell counts close to the 4-cell and 12-cell cutoffs described in **Interpretation of Results**.

Reproducibility of Bladder Carcinoma Cell Culture Specimens

To assess the reproducibility of the UroVysion assay, analyses of the signal distributions for CEP 3, CEP 7, CEP 17, and LSI 9p21 were assessed for inter-site (4) reproducibility on slides prepared from 4 different bladder carcinoma cell lines. Four specimens prepared from human bladder carcinoma cell lines with normal (1 specimen) and abnormal (3 specimens) signal distributions were evaluated for CEP 3, CEP 7, CEP 17, and LSI 9p21 according to the instructions for analysis of quality control slides in this package insert (see Interpretation of Results: Analysis of Quality Control Slides). Each site assayed 4 replications of the same specimen on each of 4 assay days (a different specimen each day), using a single probe lot for all specimens. On each assay day, an additional "wild card" specimen was added to eliminate bias and was not included in the data analysis. Each specimen was evaluated by 1 observer at each site. Informative results were obtained in 95.0% (76/80) of the specimens on the first attempt. Hybridization of all replacement slides was successful.

The mean, standard deviation, and percent CV of the average number of signals for the 4 probes is shown in **Table 3**. As shown in this table, the mean number of signals for each probe varies within a narrow range. The absence of LSI 9p21 signals in specimen 2 causes a large %CV for this probe, but this specimen is still easily classified as having a loss of the 9p21 locus; in 95% of the observations on this specimen (19/20) the average number of LSI 9p21 signals was < 0.2.

There were no false negative results in this study of human bladder carcinoma cell lines; all (48/48) evaluations of specimens 2, 3, and 4 (16 each) would have been classified as positive by the definition of ≥ 4 cells with gains of multiple chromosomes (3 or more signals for 2 or more of CEP 3, CEP 7, or CEP 17), or ≥ 12 cells with homozygous loss of 9p21 (0 LSI 9p21 signals). Of the 16 evaluations of the normal specimen, 1 would have been classified as positive using the above definition; this case showed 6 cells with gains of multiple chromosomes.

Table 3. Between-Site Reproducibility

		Number of Signals			
Specimen	**Statistics**	**CEP 3**	**CEP 7**	**CEP 17**	**LSI 9p21**
	Mean	2.21	2.12	2.14	2.19
	S.D.	0.15	0.12	0.12	0.21
1	C.V. (%)	6.79%	5.52%	5.66%	9.66%
	Range	2.08-2.68	1.92-2.40	1.96-2.52	2.00-2.92
	n	16	16	16	16
	Mean	3.95	4.31	3.42	0.03
	S.D.	0.10	0.25	0.16	0.07
2	C.V. (%)	2.49%	5.76%	4.76%	220.44%
	Range	3.84-4.16	3.76-4.84	3.16-3.72	0.00-0.24
	n	16	16	16	16
	Mean	4.28	3.55	3.42	3.86
	S.D.	0.32	0.34	0.25	0.47
3	C.V. (%)	7.58%	9.47%	7.21%	12.14%
	Range	3.88-5.04	3.12-4.24	3.04-3.96	3.16-4.72
	n	16	16	16	16
	Mean	3.18	3.88	3.84	3.85
	S.D.	0.15	0.10	0.10	0.15
4	C.V. (%)	4.63%	2.45%	2.70%	3.90%
	Range	2.96-3.52	3.64-4.04	3.64-4.12	3.56-4.24
	n	16	16	16	16

Clinical Studies

Bladder Cancer Recurrence: Performance vs Standard of Care

Study Summary

A multi-center, prospective, longitudinal study was conducted at 21 sites over 17 months to further define the performance characteristics of the UroVysion Kit relative to cystoscopy followed by histology, the standard of care for monitoring for disease recurrence in patients previously diagnosed with bladder cancer. The comparative reference used for all calculations was cystoscopy with histology confirmation for positive or suspicious cystoscopies. If a patient had a positive cystoscopy but histology was absent (eg, the lesion was fulgurated), then the specimen was considered positive for bladder cancer. If a test had a suspicious cystoscopy but histology was absent, then the case was omitted from analysis. A total of 309 patient visits were conducted at 21 investigation sites, resulting in 251 usable office visits. The 58 unusable visits included 17 that did not meet the eligibility criteria, 16 with insufficient urine volume, 10 with suspicious cystoscopies but no histology, and in 15 cases urine was not sent to the testing laboratories. All specimens were preserved in Carbowax. Urine processing and analysis were conducted at 1 centralized testing laboratory. The manual pretreatment and manual UroVysion assay procedures were used for all specimens. UroVysion assay and analysis on the 251 usable office visits resulted in 234 informative results, representing 176 unique patients. For patients who experienced a recurrence during the trial (as determined by cystoscopy/histology), the first positive visit was used (ie, the visit at which the diagnosis of recurrence was established). For the nonrecurring patients, the last negative visit was used for those patients with more than 1 visit. The demographics for the 176 unique patients are summarized in **Table 4**.

Table 4. Patient Demographics
Bladder Cancer Recurrence Study

Sex		
	Male	132
	Female	44
Race		
	Caucasian	153
	African American	3
	Hispanic	3
	Other	13
	Unknown	4
Age		
	Range	36-98 years
	Average	71 years

Performance vs Standard of Care

Of the eligible patients with informative UroVysion results, 62 were positive by cystoscopy/histology. A breakdown of the number of tumors by stage and grade is shown in **Table 5**.

Table 5. Number of Tumors, by Stage and Grade
Bladder Cancer Recurrence Study

	Tumor Grade					
Tumor Stage	**ND**	**1**	**2**	**3**	**Unknown**	**Total**
ND	11	0	0	0	0	11
Ta	0	20	6	6	0	32
T1	0	0	2	3	1	6
T2	0	0	0	2	1	3
Tis	0	0	0	7	0	7
Unknown	0	2	1	0	0	3
Total	11	22	9	18	2	62

ND = not assigned or no biopsy

Table 6 shows the performance of the UroVysion Kit, relative to cystoscopy/histology, by tumor stage and grade for all cases with biopsy information available. The UroVysion Kit showed greatest clinical sensitivity (100%) among the most severe tumors (T2 and Tis), when compared to cystoscopy/histology.

Table 6. Comparison of UroVysion vs Cystoscopy/Histology for Detection of Bladder Cancer Recurrence by Tumor Stage and Grade[a]

	Clinical Sensitivity (%)
Stage	
All	36/48 (75.0%)
Ta, Grade 1	11/20 (55.0%)
Ta, Grade 2,3	10/12 (83.3%)
T1	5/6 (83.3%)
T2	3/3 (100%)
Tis	7/7 (100%)
Grade	
All	36/49 (73.5%)
1	12/22 (54.5%)
2	7/9 (77.8%)
3	17/18 (94.4%)

[a] Biopsy was not performed in 11 cases. In addition, no stage was assigned in 3 cases and no grade in 2 cases.

Table 7 shows a comparison of the performance of the UroVysion Kit relative to cystoscopy followed by histology. Overall, FISH analysis with the UroVysion Kit demonstrated a clinical sensitivity of 71.0% and a clinical specificity of 65.8% when compared to the results of cystoscopy, followed by histology in the case of positive or suspicious cystoscopy

NOTE: A positive cystoscopy without a biopsy was considered positive in this analysis.

Table 7. Comparison of UroVysion vs Cystoscopy/Histology for Detection of Bladder Cancer Recurrence

UroVysion	Cysto/Histo +	Cysto/Histo -	Total
+	44	39	83
-	18	75	93
Total	62	114	176

Clinical Sensitivity = 71.0% (44/62) (95% CI = 58.1% - 81.8%)
Clinical Specificity = 65.8% (75/114) (95% CI = 56.3% - 74.4%)
Accuracy = 67.6% (119/176) (95% CI = 60.2% - 74.5%)
(+) Predictive Value = 53.0% (44/83) (95% CI = 41.7% - 64.1%)
(-) Predictive Value = 80.6% (75/93) (95% CI = 71.1% - 88.1%)
Prevalence = 35.2% (62/176) (95% CI = 28.2% - 42.8%)

The positive and negative predictive values of the UroVysion Test could be determined for prevalence rates of 10%, 20%, and 30%; these are presented in **Table 8**. This extrapolation assumed a clinical sensitivity of 71.0% and a clinical specificity of 65.8% (**Table 7**).

Table 8. Hypothetical Positive Predictive and Negative Predictive Values of the UroVysion Test

Bladder Cancer Recurrence Prevalence	PPV	NPV
10%	18.7%	95.3%
20%	34.2%	90.1%
30%	47.1%	84.1%

Table 9 shows a comparison of the performance of the UroVysion Kit relative to cystoscopy/histology in patients who had received their last treatment with intravesical BCG within 3 months of UroVysion testing. The mean time duration of BCG treatment was 1.3 months (range 0.4 to 3.4 months). The mean time between the last BCG treatment and UroVysion testing among these patients was 1.3 months; the range was 0 (treatment ongoing at the time of UroVysion testing) to 3 months. Three of the 12 true positive cases were Tis, 3 were stage Ta grade 1, 3 were stage Ta grade 3, 2 were stage T1 grade 3, and 1 was stage T2 grade 3 (muscle invasive); the 1 false negative case was stage Ta grade 1.

Table 9. Comparison of UroVysion vs Cystoscopy/Histology for Detection of Bladder Cancer Recurrence in Patients on BCG Therapy within 3 Months

UroVysion	Cysto/Histo +	Cysto/Histo -	Total
+	12	10	22
-	1	16	17
Total	13	26	39

Clinical Sensitivity = 92.3% (95% CI = 64.0% - 99.8%)
Clinical Specificity = 61.5 % (95% CI = 40.6% - 79.8%)
Accuracy = 71.8% (95% CI = 55.1% - 85.0%)
(+) Predictive Value = 54.5% (95% CI = 32.2% - 75.6%)
(-) Predictive Value = 94.1% (95% CI = 71.3% - 99.9%)
Prevalence = 33.3% (95% CI = 19.1% - 50.2%)

Longitudinal Study

As a continuation of the multi-center prospective study described above, office visit information (without UroVysion or BTA*stat* testing) was subsequently collected for patients who had not experienced a relapse (ie, cystoscopy/histology negative) for a period of approximately 1 year from their last visit during the main phase of the trial. Of the 114 eligible patients, office visit form information was collected from 105. A total of 335 patient visits were reported, resulting in 299 usable office visits, representing 104 unique patients.

NOTE: For 1 patient the only office visit reported was an ineligible visit.

The 36 unusable visits included 21 that did not meet eligibility criteria and 15 with suspicious cytoscopies but no histology. For patients who experienced a recurrence (as determined by cytoscopy/histology), the first positive visit was used. For non-recurring patients, the last negative visit was used for those patients with more than 1 visit.

The results showed recurrence in a greater percentage of patients in the UroVysion positive, cystoscopy/histology negative group than in the UroVysion negative, cystoscopy/histology negative group. The results are summarized in **Table 10**.

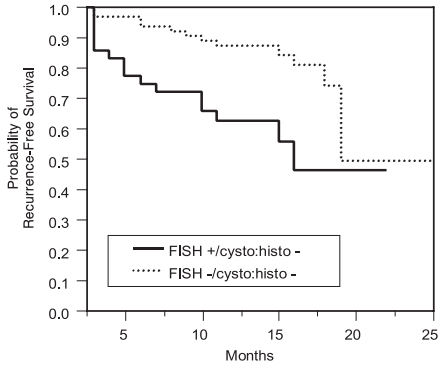
Table 10. Longitudinal Study Summary

	UroVysion - /cysto:histo -	UroVysion+ /cysto:histo -
% Recurrence	19.12% (13/68)	41.67% (15/36)
Follow-up time (months):		
No recurrence	14.3±3.9	13.5±3.4
Recurrence	11.0±5.8	6.9±4.4
Recurrence Details[a]:		
Stage		
Ta G1	5	3
Ta G2,3	0	1
T1	2	0
Tis	0	1
Grade		
1	5	5
2	1	1
3	1	1

[a] Biopsy was not performed in 8 cases (4 UroVysion+/cysto:histo-, 4 UroVysion-/cysto:histo-). Slides were not provided by collection site for assessment by the central pathologist in 6 cases (4 UroVysion+/cysto:histo-, 2 UroVysion-/cysto:histo-). No stage was assigned in 2 UroVysion+/cysto:histo- cases.

Probability estimates for nonrecurrence at various intervals were determined using the product-limit method for right-censored data (ie, Kaplan-Meier). Analysis of homogeneity between the 2 patient groups (anticipatory positives, and true negatives) was determined using the log-rank statistic. As shown in **Figure 10**, the analysis shows that a statistical difference was maintained throughout the follow-up period between the UroVysion +/cysto:histo - and the UroVysion -/cysto:histo - groups. The p-value is 0.0031. A similar analysis using the parametric Weibull considered the interval censoring directly; the difference was again significant, with p = 0.0236.

Figure 10. Recurrence-Free Survival for Patients in the UroVysion -/cysto:histo – vs UroVysion +/cysto:histo – Groups



Specificity

Study Summary

In addition to the UroVysion clinical specificities of 65.8% established in the bladder cancer recurrence study and 77.7% established in the hematuria study, a multi-center, prospective study was conducted to establish specificity of the UroVysion test in healthy subjects and urology patients without prior history or clinical evidence of bladder cancer.

A total of 315 patient visits were conducted in conjunction with this trial, resulting in 309 usable office visits. The 6 unusable visits included 1 that failed to meet the study eligibility criteria, 4 with insufficient urine volume, and in 1 case urine was not sent to the testing laboratory. All specimens were preserved in Carbowax. The manual pretreatment and manual UroVysion assay procedures were used for all specimens. Since several patients' health conditions fell into multiple categories, the 275 patient specimens yielding informative results represented 357 data points. The patient population is summarized by category in **Table 11**.

Table 11. Patient Population		
Condition	**No. of Patients**	
Healthy Subjects	59	
Non-Smokers		*50*
Smokers		*9*
Non-GU Benign Diseases	48	
Non-GU Cancer	3	
GU Diseases	184	
BPH		*58*
Microhematuria		*15*
Interstitial Cystitis		*11*
Inflammation/Infection: Other		*17*
STD		*2*
Other		*81*
GU Cancer (non-bladder)	61	
Prostate		*58*
Renal		*3*
GU Trauma	2	
Total:	**357**	

Specificity

The overall specificity of the UroVysion test in healthy subjects and urology patients without prior history or clinical evidence of bladder cancer was 93.0% (332/357). A summary of the overall specificity and the specificity by category is shown in **Table 12**. To eliminate the potential bias of including multiple data points for any particular patient, the specificity was also calculated on "unique cases", where each patient was counted only once, regardless of the number of medical conditions present. The specificity among the unique cases was 94.5% (260/275, **Table 12**).

Table 12. Summary: UroVysion Kit Specificity	
Overall Specificity	93.0% (332/357)
Unique Patients	*94.5% (260/275)*
Healthy vs Non-Healthy	
Healthy	*100% (59/59)*
Non-Healthy	*93.1% (201/216)*
Smokers vs Non-Smokers[a]	
Smokers	*95.2% (40/42)*
Non-Smokers	*94.7% (234/247)*
Individual Categories[b]	
Healthy Donors	100% (59/59)
Healthy non-smokers	*100% (50/50)*
Healthy smokers	*100% (9/9)*
Non-GU Benign Diseases	91.7% (44/48)
Non-GU Cancer[c]	66.7% (2/3)
GU Diseases	91.9% (169/184)
BPH	*91.4% (53/58)*
Microhematuria	*86.7% (13/15)*
Interstitial Cystitis	*90.7% (10/11)*
Inflammation/Infection: Other	*100% (17/17)*
STD	*100% (2/2)*
Other	*91.4% (74/81)*
GU Cancer (non-bladder)	91.8% (56/61)
Prostate	*91.4% (53/58)*
Renal	*100% (3/3)*
GU Trauma	100% (2/2)

[a] Smoking status unknown in 1 patient.

[b] Some non-healthy patients had health conditions falling into multiple disease categories, resulting in totals > 275 for individual disease categories.

[c] Non-GU cancers included breast (1), colon (1), and leukemia (1).

Based on the patient population in this study, the UroVysion test, when used with the manual pretreatment and manual UroVysion assay procedures, demonstrated an overall specificity of 93.0% (332/357), with a 100% specificity (59/59) among healthy subjects. The specificity among unique cases was 94.5% (260/275). The false positive results found in 15 patients represented the following categories (note that some patients had health conditions falling into multiple disease categories); non-genitourinary (GU) benign diseases (4), non-GU cancer (1), GU diseases (15), and GU cancer (5). These results indicate that the test is highly specific in this patient group and that the UroVysion probes reacted only with the intended chromosomes.

Symptomatic Patients: Performance vs Standard of Care

Study Summary

A multi-center, prospective, longitudinal study was conducted to further define the performance characteristics of the UroVysion Kit relative to cystoscopy followed by histology, the standard of care for diagnosing bladder cancer in patients presenting with hematuria. The comparative reference used for all calculations was cystoscopy with histology confirmation for positive or suspicious cystoscopies. A total of 629 patient visits were consented at 23 investigation sites, resulting in 497 eligible patients. The 132 ineligible patients included: 74 that did not meet the eligibility criteria; 12 with insufficient urine volume; 14 with urine improperly shipped to the testing laboratories; 12 who initially consented but then refused entry prior to providing a urine specimen; 18 whose specimens were collected after the study end, or whose urine was not received at the testing laboratory; and 2 whose informed consent was not properly documented. Urine processing and analysis were conducted at 3 centralized testing laboratories. All specimens were preserved in PreservCyt. Two of the 3 laboratories used the manual pretreatment method; 1 site used the automated pretreatment procedure. All UroVysion assays were conducted using the automated (HYBrite) procedure. The patient demographics for the 497 eligible patients are summarized in **Table 13**.

Table 13. Patient Demographics Symptomatic Patient Study		
Sex		
	Male	298
	Female	199
Race		
	Caucasian	440
	African American	26
	Hispanic	15
	Asian	4
	Other/Unspecified	12
Age		
	Average	63.1 years
	Range	40-97 years

Performance vs Standard of Care

UroVysion assay and analysis on the 497 eligible patients resulted in 479 informative results for initial study visits. Of the 479 initial study visits with informative results; 6 had uninformative cytology results and, per protocol were not included in the analysis, leaving 473 patients in the main data set. Of the 473 eligible patients in the main data set, 50 were positive for bladder cancer by cytosocopy/histology, and 1 for ureteral cancer. A breakdown of the number of tumors by stage and grade is shown in **Table 14**.

Table 14. Number of Tumors, by Stage and Grade Symptomatic Patient Study

	Tumor Grade				
Tumor Stage	**1**	**2**	**3**	**Unknown**	**Total**
Ta	21	6	4	0	31
T1	0	3	3	1[c]	7
T2	0	1	8	1[c]	10
Tis	0	0	1[a]	0	1
Unknown	0	0	1	1[b]	2
Total	21	10	17	3	51

[a] **NOTE: Discrepant analysis by both the local pathologist and an alternate central pathologist showed no cancer.**

[b] One case whose initial cystoscopic examination was negative, but who was subsequently diagnosed with ureteral cancer within 6 months of the initial study visit.

[c] adenocarcinomas.

Table 15 shows the performance of the UroVysion Kit, relative to cystoscopy/histology, by tumor stage and grade for all positive cases.

Table 15. Comparison of UroVysion vs Cystoscopy/Histology for Detection of Bladder Cancer by Tumor Stage and Grade

	UroVysion	**Cytology**
Stage		
TaG1	48% (10/21)	24% (5/21)
TaG2	83% (5/6)	50% (3/6)
TaG3	100% (4/4)	50% (2/4)
T1	86% (6/7)	43% (3/7)
T2	90% (9/10)	60% (6/10)
Tis	0% (0/1)[b]	0% (0/1)[b]
Unknown[a]	50% (1/2)	50% (1/2)
Grade		
1	48% (10/21)	24% (5/21)
2	70% (7/10)	30% (3/10)
3	88% (15/17)	53% (9/17)
Unknown[a,c]	100% (3/3)	100% (3/3)

[a] 1 case with unknown stage (grade 3); 1 ureteral cancer of unknown stage and grade.

[b] **NOTE: Discrepant analysis by both the local pathologist and an alternate central pathologist showed no cancer.**

[c] Includes 2 adenocarcinomas (1 stage T1, 1 stage T2) with unknown grade.

Table 16 shows a comparison of the performance of the UroVysion Kit relative to cystoscopy followed by histology. Overall, FISH analysis with the UroVysion Kit demonstrated a clinical sensitivity of 68.6% and a clinical specificity of 77.7% when compared to the results of cystoscopy, followed by histology in the case of positive or suspicious cystoscopy.

Table 16. Comparison of UroVysion vs Cystoscopy/Histology for Detection of Bladder Cancer: Adenocarcinoma Cases Included

		Cysto/Histo		
UroVysion		+	-	Total
	+	35	94[b]	129
	-	16	328	344
	Total	51[a]	422	473

[a] Includes 1 case ureteral cancer.

[b] Includes 3 patients diagnosed with upper urinary tract tumors within 6 months of their study visit.

Clinical Sensitivity = 68.6% (35/51) (95% CI = 54.1% - 80.9%)
Clinical Specificity = 77.7% (328/422) (95% CI = 73.4% - 81.6%)
Accuracy = 76.7% (363/473) (95% CI = 72.7% - 80.5%)
(+) Predictive Value = 27.1% (35/129) (95% CI = 19.7% - 35.7%)
(-) Predictive Value = 95.3% (328/344) (95% CI = 92.6% - 97.3%)
Prevalence = 10.8% (51/473) (95% CI = 8.1% - 13.9%)

Thus, a negative result does not rule out all bladder cancer. Neither does a negative UroVysion result mean that an individual will never develop bladder cancer.

In addition, 3 patients, whose initial bladder cystoscopy was negative, were subsequently diagnosed with upper urinary tract tumors (pTaG3 transitional cell carcinoma of the renal pelvis; G3 invasive papillary urothelial carcinoma of the ureter plus Tis of the ureter; adenocarcinoma of the left kidney) within 6 months of this initial study visit. All 3 of these cases were positive by UroVysion; 1 of the 3 was positive by cytology.

Positive UroVysion results in the absence of other signs or symptoms of bladder cancer recurrence may be evidence of other urinary tract related cancers, eg, ureter, urethra, renal, and/or prostate in males, and further patient follow-up is justified.

The positive and negative predictive values of the UroVysion Test could be determined for prevalence rates of 1%, 3%, and 10.5%; these are presented in **Table 17**. This extrapolation assumed a clinical sensitivity of 68.6% and a clinical specificity of 77.7% (**Table 16**).

Table 17. Hypothetical Positive Predictive and Negative Predictive Values of the UroVysion Test

Bladder Cancer Prevalence	**PPV**	**NPV**
1.0%	3.1%	99.6%
3.0%	8.9%	98.9%
10.5%	27.0%	95.5%

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TECHNICAL ASSISTANCE

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