

## SUMMARY OF SAFETY AND EFFECTIVENESS DATA

### I. GENERAL INFORMATION

|  |   |
|--|---|
| Device Generic Name:                         | Fluorescence <i>in situ</i> hybridization (FISH) reagents   |
| Device Trade Name:                           | UroVysion™ Bladder Cancer Kit (UroVysion™ Kit)  |
| Applicant's Name and Address:                | Vysis, Inc. (a wholly-owned subsidiary of Abbott Laboratories)<br>3100 Woodcreek Drive<br>Downers Grove, IL 60515 |
| Premarket Approval Application Number:       | P030052   |
| Date of Panel Recommendation:                | None  |
| Date of Notice of Approval to the Applicant: | January 24, 2005  |

### II. Indications for 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.

### III. CONTRAINDICATIONS

There are no known contraindications for the UroVysion Bladder Cancer Kit

### IV. WARNINGS AND PRECAUTIONS

Refer to the product labeling for a list of warnings and precautions.

### V. DEVICE DESCRIPTION

The UroVysion 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  
Vysis P.N.: 30-171070 (20 Test); 36-171070 (100 Test)  
Quantity: 60 µL (20 Test); 300 µL (100 Test)

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. 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 four-color, four-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™, and CEP 17 SpectrumAqua™ that hybridize to the centromere regions of chromosomes 3, 7, and 17, respectively. In addition, a unique sequence probe, Locus Specific Identifier (LSI®) p16 (9p21) SpectrumGold™, is included that hybridizes to the p16 gene at 9p21.

2) DAPI II Counterstain

Quantity: 300 µL (20 Test); 1000 µL (100 Test)

Composition: 125 ng/mL DAPI (4,6-diamidino-2-phenylindole) in 1,4-phenylenediamine, glycerol, and buffer

3) NP-40

Quantity: 4 mL (2 x 2 mL)

Composition: NP-40 (non-ionic detergent)

4) 20X SSC

Quantity: 66 g for up to 250 mL of 20X SSC solution

Composition: sodium chloride and sodium citrate

In addition, the following materials and reagents are necessary to perform the assay.

Materials Required but Not Provided

Laboratory Reagents

- ProbeChek UroVysion Control slides Order No. 30-805070  
Three (3) glass microscope slides containing both a positive control and a negative control on the same slide (*i.e.*, two 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.
- FISH Specimen Pretreatment Reagent Kit (Order No. 32-801270), which includes:  
Protease (3 x 25 mg)  
Pepsin (2500-3000 units/mg)

- Pepsin Buffer (3 x 50 mL)
- 10 mM HCl
- Phosphate Buffered Saline (2 x 250 mL)
- 1X PBS
- 100X MgCl<sub>2</sub> (3 x 0.5 mL)
- 2M MgCl<sub>2</sub>
- 20X SSC (66 g)
- 10% neutral buffered formalin
- Carnoy's Fixative (3:1 (v:v) methanol:glacial acetic acid)
- Immersion oil for appropriate microscope objectives. Store at room temperature (15-30°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. Store at 4°C for up to one month from delivery (See manufacturer's recommendations for detailed information).

#### Specimen Preservation

- Carbowax (2% polyethylene glycol in 50% ethanol) Suggested source: Sigma Product #P5402
- ThinPrep™ PreservCyt™ Solution, Cytoc Corp. Product #70406-001

#### Laboratory Equipment

- Glass coverslips (12 mm round and 18 mm glass coverslips are recommended)
- 12-well, 6 mm circle microscope slides. Suggested type: Shandon Product #9991090
- Microliter pipettors (1-10 µL and 20-200 µL) and clean tips
- Conical centrifuge tubes (15 and 50 mL)
- Timer (± 1 sec.)
- 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 #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)
- Light microscope equipped with a 20X objective
- pH meter and pH paper
- Calibrated thermometer
- 0.45 µm pore filtration unit
- Desiccant
- HYBrite™ System (optional)
- VP 2000™ Processor (optional)

#### 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 sub-optimal 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  $\geq 0.75$  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 Vysis 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.

### Principle of Device Methodology

The UroVysion Kit is based upon fluorescence *in situ* hybridization (FISH) DNA probe technology. *In situ* hybridization is a technique that allows the visualization of specific nucleic acid sequences within a cellular preparation. Specifically, DNA fluorescence *in situ* hybridization (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, four-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 pre-mixed and pre-denatured 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.

## VI. ALTERNATE PRACTICES AND PROCEDURES

The current “gold standard” for the detection of bladder cancer in symptomatic patients or monitoring for recurrence of bladder cancer in patients previously diagnosed with bladder cancer is cystoscopic examination with biopsy of suspicious lesions followed by histopathology of the biopsy.

Urine cytology is also used to detect bladder cancer. However, that procedure has a low sensitivity, but high specificity for bladder cancer detection.

## **VII. MARKETING HISTORY**

The UroVysion Bladder Cancer Kit that is indicated for the detection of bladder cancer in symptomatic patients has not been marketed previously for clinical use.

The Vysis UroVysion Bladder Cancer Recurrence Kit was first cleared by the FDA on August 03, 2001 in 510(k) K011031. The UroVysion Kit has been marketed by Vysis since 2001 in the United States and in Austria, Belgium, France, Germany, Greece, Ireland, Italy, Japan, Netherlands, Nordic Region, Poland, Spain, Switzerland, Turkey, and the United Kingdom.

The UroVysion Bladder Cancer Recurrence Kit has never been withdrawn from any markets because of issues related to safety and effectiveness of the device.

## **VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH**

When the UroVysion Bladder Cancer Kit is used as indicated, the adverse effects on the health of the patients being evaluated for bladder cancer are associated with a false positive or negative test result.

A false positive result from the UroVysion Kit may lead to more aggressive follow up procedures and possibly the initiation of inappropriate therapy. A false positive result could potentially result in more frequent monitoring via the current “gold standard” cystoscopy. The potential adverse effects to the patient with more frequent cystoscopy examinations, which are considered moderately invasive, include pain and discomfort and an increased risk of infection and perforating the bladder.

Alternatively, a false negative result from the UroVysion Kit may result in a patient receiving a delayed diagnosis of either a primary or recurring tumor, and not receiving adequate treatment or monitoring. A false negative result could potentially allow a cancer to progress to a more aggressive state and possibly lead to either loss of the bladder due to the need to re-section or, in some cases, death. The likelihood of such an event is minimized because the UroVysion Kit is used in conjunction with other standard diagnostic procedures (e.g., cystoscopy).

The risks of a false positive or false negative result from the UroVysion Kit are also minimized through the use of ProbeChek UroVysion Control Slides, Product No. 30-805070, and specimen handling and pretreatment procedures that are recommended in the UroVysion Kit Package Insert. These controls are recommended for use in every run and provide information on assay reliability and performance. Moreover, the probe configuration of the UroVysion Kit functions as an internal control because the product is a four-colored probe product. Red, green, gold and aqua probes serve as additional internal controls for the other probe components and may be used by the testing laboratory to verify assay condition suitability prior to analyzing patient samples.

## IX. SUMMARY OF PRECLINICAL STUDIES

Preclinical testing of the UroVysion Kit included analytical specificity, reproducibility, interfering substances, validation of use of Cytoc PreservCyt® as a microbial inhibitory preservative/transport medium with urine specimens, validation of the performance of the UroVysion Kit using PreservCyt, stability studies to support shelf life and shipping conditions of the UroVysion Kit, and validation of the VP 2000™ Processor and HYBrite™ Denaturation/Hybridization System as an optional semiautomatic instrument system for use with UroVysion Kit.

The UroVysion Kit was the subject of two previous 510(k) submissions to garner clearance of this test for the indication of monitoring previously diagnosed bladder cancer patients. Because no changes have been made to the UroVysion Kit, the specimen type (voided urine) required to perform the assay, or to the assay procedure, the preclinical studies for analytical specificity, reproducibility, and the effect of interfering substances were not repeated for this submission. The data for the analytical specificity, reproducibility, interfering substances studies were previously submitted in two cleared UroVysion 510(k) submissions (K011031 and K013785). Thus, the preclinical studies may be divided into two parts: old and new studies.

### A. Old Preclinical Studies

#### 1. Analytical Specificity

**Objective:** The objective of this study was to establish the specificity of the UroVysion probes with their intended chromosomes in metaphase spreads

**Summary of Studies Performed:** Locus specificity studies were performed with metaphase spreads according to standard Vysis 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 testing with the four UroVysion probes.

**Results:** 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 four UroVysion probes.

**Conclusions:** These studies demonstrate that the UroVysion Kit reacts specifically with the intended chromosomes and no others, as shown by metaphase analysis. This conclusion was supported also by the 93% percent negativity of 357 normal and bladder cancer negative persons with other diseases.

#### 2. Reproducibility

**Objective:** The overall objective of all of the reproducibility studies was to establish the intra-assay, inter-site, inter-day, inter-lot, inter-observer, and the

overall reproducibility of the UroVysion™ Bladder Cancer Kit, so that these performance characteristics may be published in the package insert.

a. Reproducibility of Patient Samples

Conducting reproducibility studies on urine specimens from bladder cancer patients was requested by the FDA in the first major deficiency letter from the FDA to the sponsor dated March 16, 2004. The sponsor responded in Amendment 4 received by the FDA on August 5, 2004, that the use of real patient bladder cancer cells was not feasible for use in repeating studies because one patient cell pellet does not yield enough cells to replicate the specimen between observers. Hence the reproducibility of results on morphologically abnormal cells was not assessed.

b. Reproducibility of Bladder Carcinoma Cell Culture Specimens

Objective: The objective of the reproducibility studies was to assess the reproducibility of the signal distributions of the UroVysion CEP 3, CEP 7, CEP 17, and LSI 9p21 probes for intra-assay, inter-site (from four sites), inter-day, inter-lot, inter-observer, and overall reproducibility on slides prepared from four different human bladder carcinoma cell lines.

Summary of Studies Performed:

Four specimens prepared from human bladder carcinoma cell lines with normal (one specimen) and borderline positive abnormal (three specimens) signal distributions were evaluated repeatedly at four different sites for CEP 3, CEP 7, CEP 17 positive and LSI 9p21 negative chromosome counts according to the instructions in the UroVysion Bladder Cancer Kit package insert. Each site assayed four replications of the same specimen on each of four 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 one observer at each site.

Results: The data in Table 1 represents a summary of reproducibility over all variables (worst case reproducibility). As can be seen very good coefficients of variation (CVs) were still demonstrated.

Table 1. Between-Site Reproducibility

| Specimen | Statistics | Number of Signals |       |        |          |
|----------|------------|-------------------|-------|--------|----------|
|          |            | CEP 3             | CEP 7 | CEP 17 | LSI 9p21 |
| 1        | Mean       | 2.21              | 2.12  | 2.14   | 2.19     |
|          | S.D.       | 0.15              | 0.12  | 0.12   | 0.21     |
|          | 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        |
| 2 | Mean     | 3.95      | 4.31      | 3.42      | 0.03      |
|   | S.D.     | 0.10      | 0.25      | 0.16      | 0.07      |
|   | 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        |
| 3 | Mean     | 4.28      | 3.55      | 3.42      | 3.86      |
|   | S.D.     | 0.32      | 0.34      | 0.25      | 0.47      |
|   | 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        |
| 4 | Mean     | 3.18      | 3.88      | 3.84      | 3.85      |
|   | S.D.     | 0.15      | 0.10      | 0.10      | 0.15      |
|   | 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        |

As can be seen in Table 1, there were no false negative results in this study; all (48 of 48) evaluations of abnormal specimens 2, 3 and 4 (16 each) would have been classified as positive by the definition of  $\geq 4$  cells with gains of multiple chromosomes, or  $\geq 12$  cells with homozygous loss of 9p21. Of the 16 evaluations of the normal specimen, one would have been classified as positive using the above definition; this case showed 6 cells with gains of multiple chromosomes.

Conclusions: The UroVysion Kit is highly reproducible in the vicinity of the test cutoff even between laboratories and provides reliable information with respect to CEP 3, CEP 7, CEP 17, and LSI 9p21 signal distribution in human bladder carcinoma cell culture specimens. The FDA requested additional reproducibility studies using cell lines containing higher numbers of chromosomes. The sponsor responded in Amendment 4 received by the FDA on August 5, 2004 that cell lines with higher numbers of chromosomes could not be found. They justified their reproducibility studies by stating that the reproducibility around the cutoff of a qualitative test is the most important level to test. The FDA accepted the sponsor's justification of their reproducibility studies.

### 3. Interference

Objective: The objective of this study was to determine whether common urine constituents, microbial contaminants, therapeutic agents, and/or laboratory preservatives interfere with performance of the UroVysion Kit.

### Summary of Studies Performed:

Three human urine pools prepared from voided urine specimens were obtained from normal donors. Each of the 25 substances under investigation was spiked into aliquots of each of the three pools at two different concentrations. The highest concentration tested is given in Table 2 below. Six preservatives commonly used for urine cytology were also tested at the standard concentration, using the automated pretreatment and automated UroVysion assay procedures.

Table 2. Substances Tested for Assay Interference

| Substance                              | Highest Concentration Tested |
|--|------------------------------|
| <i>Possible Urine Constituents</i>     |                              |
| 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)                | $1 \times 10^6$ cells/mL     |
| White Blood Cells (human)              | $1 \times 10^6$ cells/mL     |
| Sodium Chloride                        | 730 mg/dL                    |
| Uric Acid                              | 250 mg/dL                    |
| Caffeine                               | 117 mg/dL                    |
| Ethanol                                | 1% (v/v)                     |
| Nicotine                               | 28 mg/dL                     |
| <i>Possible Microbial Contaminants</i> |                              |
| Candida albicans                       | $2.5 \times 10^{10}$ CFU/mL  |
| Escherichia coli                       | $2.5 \times 10^{10}$ CFU/mL  |
| Pseudomonas aeruginosa                 | $2.5 \times 10^{12}$ CFU/mL  |
| <i>Therapeutic Agents</i>              |                              |
| 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                     |
| <i>Preservatives</i>                   |                              |
| Vysis, Inc. standard: 2%               | 2% Carbowax/50% ethanol      |

| Substance                   | Highest Concentration Tested                      |
|-----------------------------|---|
| Carbowax                    | solution<br>(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 (Cytoc) | 50/50 with urine                                  |
| 100% Ethanol                | 50/50 with urine                                  |

Results: No interference was detected from any of the 25 substances and 6 preservatives tested with the UroVysion Kit. All substances and preservatives performed within the acceptance criteria of  $\pm 2$  Standard Deviations or 20% of the means

Conclusion: The data demonstrate that common urine constituents, microbial contaminants, therapeutic agents, and/or laboratory preservatives do not interfere with performance of the UroVysion Kit.

## B. New Preclinical Studies

### 1. Cytoc PreservCyt<sup>®</sup> Microbial Challenge Study with the UroVysion Bladder Cancer Kit

Objective: The objective of this study was to determine the performance of Cytoc PreservCyt (PC) as a microbial inhibitory preservative in urine spiked to known microbial concentrations, and to compare the test performance using PreservCyt with that of the previously recommended urine preservative, Carbowax<sup>™</sup> (CW) on the same microbial urine samples. Cytoc PreservCyt is a commercially available specimen transport medium manufactured by Cytoc Corporation.

#### Summary of Studies Performed:

Cells from the bladder carcinoma cell line UM-UC-3 (which is used as a positive control in ProbeChek UroVysion Control Slides) were spiked into pooled urine specimens obtained from donors with no history of bladder cancer. PreservCyt or Carbowax was added to aliquots of the spiked urine pools, and each aliquot was inoculated with one of the following microbial organisms: *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), *Enterococcus*, or *Candida albicans* (*C. albicans*). The inoculated urine samples were stored at 2 to 8°C and 20 to 25°C for up to 72 hours.

PreservCyt was determined to be acceptable as a preservative if there was no increase greater than 13,000 CFU/mL from the initial inoculation up to 72 hours when stored at both 2 to 8°C and 20 to 25°C. The effect of PreservCyt on the performance of the UroVysion assay was determined by counting the number of signals per nucleus of the PreservCyt samples for each probe. Automated pretreatment and automated UroVysion assay procedures were used.

Results: None of the microbial challenge organisms demonstrated an increase in microbial growth from 0 to 72 hours when stored with the PreservCyt or Carbowax preservative, and acceptable UroVysion assay performance results were obtained with urine samples containing both PreservCyt and Carbowax.

Conclusion: The data demonstrate that the PreservCyt performs acceptably as a microbial inhibitory preservative in urine and that acceptable UroVysion assay performance results were obtained with urine samples containing spiked tissue culture cells using both PreservCyt and Carbowax. The acceptability of the test used with these preservatives on patient samples has not been demonstrated.

## 2. Validation of Cytoc PreservCyt Preservative for Use with the UroVysion Bladder Cancer Kit

Objective: The objective of this study was to determine the hybridization quality of the UroVysion Bladder Cancer Kit on urine specimens using PreservCyt preservative relative to Carbowax preservative, which was cleared for use in K011031 and K013785, for the purpose of establishing an alternative, commercially available preservative and transport medium for use with the UroVysion Kit. [Cytoc PreservCyt is manufactured by Cytoc Corporation.]

Summary of Studies Performed: Urine specimens from donors with no history of bladder cancer were divided into three categories: no preservative (urine only), Carbowax preservative, or PreservCyt preservative. Half of each urine sample was spiked with a bladder carcinoma cell line, and the other half remained unspiked. Samples were diluted with preservative and placed in simulated shipping conditions for up to seven days, and tested with the UroVysion Kit, using the automated pretreatment and automated UroVysion procedures. The average number of signals per nucleus for each probe was calculated for each sample. The performance of PreservCyt was considered acceptable if the average percent variation of the signals per nucleus was less than 50 between PreservCyt and Carbowax. The qualitative results and the quality of staining were also evaluated

Results: The average number of signals per nuclei, the overall classification of positive vs, negative results and the evaluation of the quality of staining demonstrated that the PreservCyt can be used as an alternative to Carbowax to preserve the specimen for the UroVysion Bladder Cancer Test.

Conclusion: The results of this study demonstrated that PreservCyt and Carbowax were equivalent in the preservation of normal urine cells for analysis by the UroVysion assay, and that PreservCyt can be used as an alternative, commercially available preservative and transport medium for use with the UroVysion Kit.

### 3. Stability of the UroVysion Bladder Cancer Kit

Objective: The objectives of this study were to determine the real time stability of the UroVysion Kit at the recommended storage condition of -20°C and to demonstrate the efficacy of these reagents following shipping simulation and freeze/thaw challenge.

Summary of Studies Performed: Real time stability testing of three lots of the UroVysion Kit stored at the recommended storage condition of -20°C was conducted at four separate time points (days 0, 180, 360, and 540) and tested on three of the following four specimen types: normal lymphocyte cells, uncultured amniocyte preparations, urine cells from a 50 year old (or older) donor or UroVysion ProbeChek control slides. All specimens were tested with test reagents and already approved reagents for comparison.

In addition, freeze-thaw stability was conducted by subjecting the UroVysion Kit to 20 freeze thaw cycles (the number of freeze/thaw cycles was equal to the number of assays for which reagents are provided), and tested at day 540 (18 months). Simulated shipping was conducted by packing the UroVysion Kit on ice packs and on ice packs placed in a 37°C incubator, and tested at day 540. The challenged kits were then tested using the manual pretreatment and automated UroVysion procedures.

Results: The results of the real time (-20°C) stability studies demonstrated that the components of the UroVysion Kit gave similar results through 540 days (18 months), even after 20 freeze/thaws and simulated shipping conditions.

Conclusion: These data support the 12 month shelf life that is currently assigned to the UroVysion Kit.

### 4. Validation of the VP 2000™ Processor and HYBrite™ Denaturization/Hybridization System for Use with the UroVysion Bladder Cancer Kit

Objective: The objective of this study was to determine if the results obtained using the recommended manual specimen pretreatment protocol and assay for the UroVysion Bladder Cancer Kit were the same whether performed manually by technician or semiautomatically using the VP 2000 Sample Processor and HYBrite™ instruments.

Summary of Studies Performed: Samples from the Interference study were used for this study. Three human urine pools prepared from voided urine specimens

were obtained from normal donors. Each of the 25 substances under investigation was spiked into aliquots of each of the three pools at two different concentrations. Thirty one interfering substances (26 compounds and six preservatives) were spiked into aliquots of each of the three urine pools and tested on three VP2000 and three HYBrite instruments. The average number of signals per nucleus for each probe was calculated for each sample. The results were compared to the manual assay results from the Interference study.

Results: The 31 substances tested produced equivalent results using the UroVysion Kit for all concentrations tested and across all six instruments. All substances and preservatives performed within 2SD or 20% of the control pools.

Conclusion: The data from this study demonstrated that the manual and semiautomated methods were equivalent (see Table 3).

Table 3. Validation of the VP2000 Processor/HYBrite System  
Manual versus Semi-Automation Study Results

| Substance                       | Concentrations   | Results     |
|---------------------------------|--|-------------|
| Possible Urine Constituents     |  |             |
| Albumin                         | 0.5 g/dL and 1.0 g/dL  | Equivalent  |
| Ascorbic Acid                   | 2.5 g/dL and 5 g/dL  | Equivalent. |
| Bilirubin (unconjugated)        | 1 mg/mL and 2 mg/mL  | Equivalent  |
| Hemoglobin                      | 50 mg/dL and 100 mg/dL                                       | Equivalent  |
| IgG                             | 5 mg/dL and 10 mg/dL   | Equivalent  |
| Red Blood Cells (human)         | $5 \times 10^5$ cells/mL and $1 \times 10^6$ cells/mL        | Equivalent  |
| White Blood Cells (human)       | $5 \times 10^5$ cells/mL and $1 \times 10^6$ cells/mL        | Equivalent  |
| Sodium Chloride                 | 365 mg/dL and 730 mg/dL                                      | Equivalent  |
| Uric Acid                       | 125 mg/dL and 250 mg/dL                                      | Equivalent  |
| Caffeine                        | 58.5 mg/dL and 117 mg/dL                                     | Equivalent  |
| Ethanol                         | 0.5% (v/v) and 1% (v/v)                                      | Equivalent  |
| Nicotine                        | 14 mg/dL and 28 mg/dL  | Equivalent  |
| Possible Microbial Contaminants |  |             |
| <i>Candida albicans</i>         | $1.25 \times 10^{10}$ CFU/mL and $2.5 \times 10^{10}$ CFU/mL | Equivalent  |
| <i>Escherichia coli</i>         | $1.25 \times 10^{10}$ CFU/mL and $2.5 \times 10^{10}$ CFU/mL | Equivalent  |
| <i>Pseudomonas aeruginosa</i>   | $1.25 \times 10^{10}$ CFU/mL and $2.5 \times 10^{12}$ CFU/mL | Equivalent  |
| Therapeutic Agents              |  |             |
| Acetaminophen                   | 2.6 g/dL and 5.2 g/dL  | Equivalent  |
| Acetylsalicylic Acid            | 2.6 g/dL and 5.2 g/dL  | Equivalent  |
| Ampicillin                      | 300 mg/dL and 600 mg/dL                                      | Equivalent  |
| BCG                             | 10 mg/dL and 20 mg/dL  | Equivalent  |
| Doxorubicin-HCl                 | 5 mg/dL and 10 mg/dL   | Equivalent  |
| Mitomycin C                     | 5 mg/dL and 10 mg/dL   | Equivalent  |

|                                      |   |            |
|--------------------------------------|---|------------|
| Nitrofurantoin                       | 25 mg/dL and 50 mg/dL   | Equivalent |
| Phenazopyridine-HCl                  | 100 mg/dL and 200 mg/dL   | Equivalent |
| Thiotepa                             | 5 mg/dL and 10 mg/dL  | Equivalent |
| Trimethoprin                         | 25 mg/dL and 50 mg/dL   | Equivalent |
| Preservatives                        |   |            |
| Vysis, Inc. standard:<br>2% Carbowax | 2% Carbowax/50% ethanol solution (33 mL<br>urine with 17 mL preservative) | Equivalent |
| UroCor, Inc. fixative                | 50/50 with urine  | Equivalent |
| CytRichRed (Autocyte)                | 50/50 with urine  | Equivalent |
| Saccamono's solution                 | 50/50 with urine  | Equivalent |
| PreservCyt solution (Cytec)          | 50/50 with urine  | Equivalent |
| 100% Ethanol                         | 50/50 with urine  | Equivalent |

## X. SUMMARY OF CLINICAL STUDIES

The UroVysion Kit was the subject of two previous 510(k) submissions (K011031 and K013785) to garner clearance of this test for the indication of monitoring previously diagnosed bladder cancer patients. This premarket approval submission is concerned only with the claim to aid in the diagnosis of bladder cancer in patients with hematuria. Hence, I will summarize only the new studies concerning diagnosis of bladder cancer that were added to the previously marketed product labeling.

### A. Prospective Study of the UroVysion™ Bladder Cancer Kit in Patients with Hematuria Suspected of Having Bladder Cancer

#### 1. Objectives

The objectives of this clinical study were to assess the safety and effectiveness of the Vysis UroVysion Kit to aid in the diagnosis of bladder cancer. This was accomplished by determining and publishing in the product labeling the expected results of the UroVysion Kit when used in a population of persons with hematuria and suspected of having bladder cancer.

#### 2. Study Design

A multi-center, prospective, blinded study was conducted to evaluate the performance of the UroVysion Kit, relative to cystoscopy followed by histology (the reference clinical diagnosis), for the detection of bladder cancer in patients with gross or microscopic hematuria and no prior history of bladder cancer. Twenty-three collection sites, one centralized pathology laboratory, and three centralized testing sites participated in this clinical study. The comparative reference used for all calculations was cystoscopy with histology confirmation for positive or suspicious cystoscopies. Investigators were blinded to the UroVysion and cytology results until after cystoscopy and/or biopsy of suspicious lesions.

The primary acceptance criterion for this trial was that the cancer rate in the patient group that tests positive be significantly greater than the rate in the group that tests negative.

The secondary acceptance criterion was that the negative predictive value (NPV) would be greater than 98%, which is (1-the historical disease prevalence rate). This criterion was based on the expected prevalence of 3%.

### 3. Patient Assessment

After obtaining written consent, patients with confirmed gross or microscopic hematuria were enrolled. Patient information was collected with respect to patient demographics, relevant health history, and cystoscopy and, if applicable, histology results. Prior to cystoscopy examination because of hematuria, voided urine was collected, preserved in PreservCyt™, and sent to the centralized testing laboratories for analysis by UroVysion and cytology. Two of the three laboratories used the manual pretreatment method; one site used the automated pretreatment procedure. All UroVysion assays were conducted using the automated (HYBrite™) procedure. In the event the patient underwent a bladder biopsy, hematoxylin and eosin (H&E) slides were submitted to the study pathologist for review. The reference clinical diagnosis by cystoscopy followed by histological examination of the tissue was the outcome measure to differentiate whether the UroVysion Kit gave a correct result or not.

### 4. Demographic Data

A total of 629 patients were consented in conjunction with this trial, 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. The patient demographics for the 497 eligible patients are summarized in Table 4, below.

Table 4. Patient Demographics

|                  |     |
|------------------|-----|
| Sex              |     |
| Male             | 298 |
| Female           | 199 |
| Race             |     |
| Caucasian        | 440 |
| African American | 26  |
| Hispanic         | 15  |



|                   |                |
|-------------------|----------------|
| Asian             | 4              |
| Other/Unspecified | 12             |
| Age               |                |
| Range             | 40 to 97 years |
| Average           | 63.1 years     |

## 5. Data Analysis and Results

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 cystoscopy/histology, and 1 for ureteral cancer. 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  
Symptomatic Patient Study

| Tumor Stage | Tumor Grade |    |                |                | Total |
|-------------|-------------|----|----------------|----------------|-------|
|             | 1           | 2  | 3              | Unknown        |       |
| Ta          | 21          | 6  | 4              | 0              | 31    |
| T1          | 0           | 3  | 3              | 1 <sup>#</sup> | 7     |
| T2          | 0           | 1  | 8              | 1 <sup>#</sup> | 10    |
| Tis         | 0           | 0  | 1 <sup>^</sup> | 0              | 1     |
| Unknown     | 0           | 0  | 1              | 1 <sup>*</sup> | 1     |
| Total       | 21          | 10 | 17             | 3              | 51    |

<sup>^</sup>Note: Discrepant analysis by both the local pathologist and an alternate central pathologist showed no cancer.

<sup>\*</sup>1 case whose initial cystoscopic examination was negative, but who was subsequently diagnosed with ureteral cancer within 6 months of the initial study visit.

<sup>#</sup> adenocarcinomas

Table 6 shows the performance of the UroVysion Kit, relative to cystoscopy/histology, by tumor stage and grade for all positive cases.

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

| Stage | UroVysion   | Cytology   |
|-------|-------------|------------|
| 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)^   | 0% (0/1)^  |
| Unknown*  | 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*# | 100% (3/3)  | 100% (3/3) |

\*1 case with unknown stage (grade 3); 1 ureteral cancer of unknown stage and grade.

^ **Note:** Discrepant analysis by both the local pathologist and an alternate central pathologist showed no cancer.

# includes 2 adenocarcinomas (1 stage T1, 1 stage T2) with unknown grade

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 sensitivity of 68.6% and a specificity of 77.7% when compared to the results of cystoscopy, followed by histology in the case of positive or suspicious cystoscopy.

Table 7. Comparison of UroVysion vs. Cystoscopy/Histology for Detection of Bladder Cancer: Adenocarcinoma Cases Included

|           |       | Cysto/Histo |     |       |
|-----------|-------|-------------|-----|-------|
| UroVysion |       | +           | -   | Total |
|           | +     | 35          | 94^ | 129   |
|           | -     | 16          | 328 | 344   |
|           | Total | 51*         | 422 | 473   |

*\*Includes one case ureteral cancer*

*^Includes 3 patients diagnosed with renal cancer within 6 months of their study visit.*

Clinical Sensitivity = 68.6% (95% CI = 54.1% - 80.9%)

Clinical Specificity = 77.7% (95% CI = 73.4% - 81.6%)

Accuracy = 76.7% (95% CI = 72.7% - 80.5%)

(+) Predictive Value = 27.1% (95% CI = 19.7% - 35.7%)

(-) Predictive Value = 95.3% (95% CI = 92.6% - 97.3%)

Prevalence = 10.8% (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 renal cancer within 6 months of this initial study visit. All 3 of these cases were positive by UroVysion; one 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, e.g., 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 8. This extrapolation assumed a clinical sensitivity of 68.6% and a clinical specificity 77.7% (Table 7).

Table 8. 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% |

B. A Satellite Study to Protocol 99-401 to Investigate the Analytical Specificity of the UroVysion™ Bladder Kit

This study was undertaken as a part of the first 510(k) submission, but is a pertinent performance characteristic, even when the test is used for diagnosis.

1. Objectives

The objective of this study was to establish the specificity of the UroVysion Kit in a population of presumed UroVysion negative healthy volunteers and urology patients without prior history or clinical evidence of bladder cancer by determining the rate of false positive results in these two populations.

2. Study Design

This was a multicenter, prospective study to determine UroVysion Kit false positive results in urine from presumed UroVysion negative healthy volunteers and urology patients without prior history or clinical evidence of bladder cancer.

3. Patient Assessment

After obtaining written consent, healthy patients and those without prior history or clinical evidence of bladder cancer were enrolled. Patient information was collected with respect to patient demographics, and relevant health history. Voided urine was collected, preserved in Carbowax and sent to the centralized testing laboratories for analysis by UroVysion. The manual pretreatment and manual UroVysion assay procedures were used for all specimens.

4. Demographic Data

A total of 315 patient visits were conducted in conjunction with this trial, resulting in 309 usable office visits. The six unusable visits included one that failed to meet the study eligibility criteria, four with insufficient urine volume, and, in one case, urine was not sent to the testing laboratory. The patient population is summarized by category in Table 9. Since several patients' health conditions fell into multiple categories, the 275 patient specimens yielding informative results represented 357 data points.

Table 9. Patient Population: UroVysion Specificity Study

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

## 5. Data Analysis and Results

The overall specificity of the UroVysion Kit in healthy subjects and urology patients without prior history or clinical evidence of bladder cancer was 93.0% (332 of 357). This was calculated using the number of (false) positive results obtained in this presumed negative group of persons. A summary of the overall specificity and the specificity by category is shown in Table 10. 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 of 275, Table 10).

Table 10. Summary: UroVysion Kit Specificity

|                                      |                        |
|--------------------------------------|------------------------|
| Overall Specificity                  | 93.0% (332/357)        |
| <i>Unique Patients</i>               | <i>94.5% (260/275)</i> |
| Healthy vs. Non-Healthy              |                        |
| <i>Healthy</i>                       | <i>100% (59/59)</i>    |
| <i>Non-Healthy</i>                   | <i>93.1 (201/216)</i>  |
| Smokers vs. Nonsmokers <sup>1</sup>  |                        |
| <i>Smokers</i>                       | <i>95.2% (40/42)</i>   |
| <i>Non-Smokers</i>                   | <i>94.7% (234/247)</i> |
| Individual Categories <sup>2</sup>   |                        |
| Healthy Donors                       | 100% (59/59)           |
| <i>Healthy non-smokers</i>           | <i>100% (50/50)</i>    |
| <i>Healthy smokers</i>               | <i>100% (9/9)</i>      |
| Non-GU Benign Diseases               | 91.7% (44/48)          |
| Non-GU Cancer <sup>3</sup>           | 66.7% (2/3)            |
| GU Diseases                          | 91.9% (169/184)        |
| <i>BPH</i>                           | <i>91.4% (53/58)</i>   |
| <i>Microhematuria</i>                | <i>86.7% (13/15)</i>   |
| <i>Interstitial Cystitis</i>         | <i>90.7% (10/11)</i>   |
| <i>Inflammation/Infection: Other</i> | <i>100% (17/17)</i>    |
| <i>STD</i>                           | <i>100% (2/2)</i>      |
| <i>Other</i>                         | <i>91.4% (74/81)</i>   |
| GU Cancer (non-bladder)              | 91.8% (56/61)          |
| <i>Prostate</i>                      | <i>91.4% (53/58)</i>   |
| <i>Renal</i>                         | <i>100% (3/3)</i>      |
| GU Trauma                            | 100% (2/2)             |

<sup>1</sup>Smoking status unknown in 1 patient.

<sup>2</sup>Some non-healthy patients had health conditions falling into multiple disease categories, resulting in totals > 275 for individual disease categories.

<sup>3</sup>Non-GU cancers included breast (1), colon (1), and leukemia (1)

Based on the patient population in this study, the UroVysion Kit demonstrated an overall specificity of 93.0% (332 of 357), with a 100% specificity (59 of 59) among healthy patients. The specificity among unique cases was 94.5% (260 of 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 (3), non-GU cancer (2), 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.

## 6. Device Failures (Hybridization Efficiency) Summarized from all Studies

### Summary of Studies Performed:

During all of the studies performed for the UroVysion Bladder cancer Kit, the hybridization efficiency based on the following definition

$$\% \text{ Hybridization Efficiency} = 100 - [\text{hybridization failures} / (\text{informative results} + \text{hybridization failures})] \times 100$$

was monitored. The following studies were monitored: quality control slides from all clinical studies, reproducibility studies with specimens prepared from human bladder carcinoma cell lines; studies conducted in urine specimens from normal persons and patients with other diseases and no evidence of bladder cancer; repeated assays; clinical study on patients with a history of bladder cancer; clinical study using automated assay procedure on patients with hematuria suspected of having bladder cancer.

Results: On the ProbeChk™ 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% (95% CI: 89.3%, 99.2%).

In a 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% (95% CI: 88.8%, 95.6%), based on the definition above. The remaining non-informative assays were the result of poor

specimen quality (e.g., insufficient number of cells) or technical error (e.g., 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% (95% CI: 81.6%, 91.4%), based on the definition above. The remaining non-informative assays were the result of poor specimen quality (e.g., insufficient number of cells) or technical error (e.g., broken slide).

Repeat assays were conducted manually on 70 specimens; six 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% (95% CI: 97.8%, 99.7%), based on the definition above. The remaining non-informative assays were the result of poor specimen quality (e.g., insufficient number of cells) or technical error (e.g., broken slide or QC slide failure). On the subset of 44 specimens for which the automated pretreatment procedure was also used, the hybridization efficiency was 96.7% (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.



Conclusion of Device Failures from all Studies: Thus, under these conditions, which simulate the normal clinical practice, the hybridization efficiency was found to be  $\geq 87\%$ . The studies showed also that hybridization efficiency between specimens processed using the manual versus automated procedures were equivalent.

## **XI. CONCLUSIONS DRAWN FROM THE STUDIES**

The results of the preclinical and clinical testing performed with the UroVysion™ Bladder Cancer Kit demonstrated that this product is reproducible and is specific for chromosomes 3, 7, 17, and 9p21 locus with analytical and clinical performance characteristics appropriate for use as an aid in the diagnosis of bladder cancer.

### **A. Risk/Benefit Analysis**

Since the UroVysion™ Bladder Cancer Kit is not intended for use as a diagnostic tool without other clinical and diagnostic data, patients will not be treated solely on the basis of results of this test. The physician will use this test to help determine the need for more or less aggressive methods and will base treatment decisions on the outcome of currently accepted standard of practice such as cystoscopic examination or imaging procedures. Therefore the risk to the patient of inappropriate or inadequate treatment based on the UroVysion™ Bladder Cancer assay is low, but the benefit of identifying patients' early malignancy is increased. Therefore, it is reasonable to conclude that the benefits of use of the device for the target population outweigh the risk of illness or injury when used as indicated in accordance with the directions for use.

### **B. Safety**

As a diagnostic test, the UroVysion Bladder Cancer Kit involves testing voided urine specimens. Such specimens are routinely collected in physicians' offices for various diagnostic tests. The UroVysion Kit specimen, therefore, presents no additional safety hazard to the patient being tested.

The UroVysion™ Bladder Cancer Kit is an *in vitro* diagnostic test and does not contact the patient. Instructions for the safe use of the product are included in the package insert.

Since the UroVysion Kit is not intended for use as a diagnostic tool without other clinical and diagnostic data, patients will not be treated solely on the basis of results of this test. The physician will use this test to help determine the need for more or less aggressive methods and will base treatment decisions on the outcome of currently accepted standard of practice such as cystoscopic examination or imaging procedures. Therefore the risk to the patient of inappropriate or inadequate treatment based on the UroVysion™ Bladder Cancer assay is low,

### C. Effectiveness

The results of testing performed with the UroVysion Kit indicated that the assay is effective as an aid for the detection of bladder cancer in patients with gross or microscopic hematuria. The UroVysion test has sensitivity and specificity comparable to the other medical device legally marketed to aid in the diagnosis of bladder cancer. Neither device has performance sufficiently strong for stand alone use, but must be used in conjunction with and not in lieu of current standard diagnostic procedures. CDRH has concluded that this device meets the statutory requirement for reasonable safety and effectiveness for the stated indication.

## XII. PANEL RECOMMENDATIONS

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Immunology Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

## XIII. CDRH DECISION

CDRH issued an approval order for the applicant's UroVysion™ Bladder Cancer Kit on January 24, 2005.

The applicant's manufacturing facility was inspected in February 17, 2004 to March 1, 2004 and found to be in compliance with the Quality System Regulation (21 CFR 820).

## XIV. APPROVAL SPECIFICATIONS

Directions for use: See labeling

Hazards to Health from Use of the Device: See Indications, Warnings and precautions in the labeling.