

| Percent Distribution of NMP22 (U/mL) Levels |     |       |        |        |        |         |      |
|---------------------------------------------|-----|-------|--------|--------|--------|---------|------|
|                                             | N   | 0-7.5 | 7.5-10 | >10-20 | >20-50 | >50-100 | >100 |
| <b>Healthy Subjects</b>                     |     |       |        |        |        |         |      |
| <b>Males &gt; 50</b>                        | 111 | 88.3% | 5.4%   | 4.5%   | 0.9%   | 0.9%    | 0%   |
| <b>Females &gt; 50</b>                      | 218 | 85.8% | 5.5%   | 6.0%   | 2.8%   | 0%      | 0%   |
| <b>Total</b>                                | 329 | 86.6% | 5.5%   | 5.5%   | 2.1%   | 0.3%    | 0%   |
| <b>Benign Disease</b>                       |     |       |        |        |        |         |      |
| UTI/Cystitis                                | 58  | 69.0% | 12.1%  | 8.6%   | 5.2%   | 5.2%    | 0%   |
| Urinary calculi                             | 71  | 64.8% | 8.5%   | 8.5%   | 9.9%   | 4.2%    | 4.2% |
| BPH/prostatitis                             | 164 | 81.7% | 4.9%   | 7.3%   | 2.4%   | 1.8%    | 1.8% |
| Other Benign Conditions                     | 259 | 78.0% | 7.7%   | 7.3%   | 2.3%   | 1.5%    | 3.1% |
| <b>Total Benign Disease*</b>                | 448 | 77.2% | 7.8%   | 7.4%   | 3.3%   | 1.3%    | 2.9% |
| <b>Other Cancers</b>                        |     |       |        |        |        |         |      |
| GI Tract                                    | 11  | 100%  | 0%     | 0%     | 0%     | 0%      | 0%   |
| Leukemia/lymphoma                           | 5   | 80.0% | 20.0%  | 0%     | 0%     | 0%      | 0%   |
| Prostate                                    | 21  | 90.5% | 4.8%   | 0%     | 4.8%   | 0%      | 0%   |
| Renal                                       | 1   | 0%    | 0%     | 0%     | 100%   | 0%      | 0%   |
| Ovarian/Cervical                            | 11  | 90.9% | 9.1%   | 0%     | 0%     | 0%      | 0%   |
| <b>Total Other Cancers</b>                  | 49  | 89.8% | 6.1%   | 0%     | 4.1%   | 0%      | 0%   |

\*Some patients are included in more than one category

The following table shows the percent distribution of the NMP22 test results for final diagnoses of the 769 patients who had symptoms or risk factors for TCC.

| Percent Distribution of NMP22 Levels (U/mL) |                              |       |        |        |        |         |       |
|---------------------------------------------|------------------------------|-------|--------|--------|--------|---------|-------|
|                                             | N                            | 0-7.5 | 7.5-10 | >10-20 | >20-50 | >50-100 | >100  |
| Risk Factor patients                        | No Urinary Tract Disease     | 265   | 79.2%  | 8.3%   | 9.1%   | 2.8%    | 0.4%  |
|                                             | Benign Urinary Tract Disease | 448   | 77.2%  | 7.8%   | 7.4%   | 3.3%    | 1.3%  |
| TCC                                         | 56                           | 37.5% | 10.7%  | 12.5%  | 16.1%  | 7.1%    | 16.1% |

The following table shows sensitivity and specificity of the NMP22 test for this study for TCC using a cut-off of  $\geq 7.5$  U/mL.

| NMP22 Results: Sensitivity, Specificity, PPV and NPV for Risk factor Patients (T0-T4) |                            |                            |                    |                    |
|---------------------------------------------------------------------------------------|----------------------------|----------------------------|--------------------|--------------------|
|                                                                                       | Sensitivity (95% Exact CI) | Specificity (95% Exact CI) | PPV (95% Exact CI) | NPV (95% Exact CI) |
| NMP22* (cut-off $\geq 7.5$ U/mL)                                                      | 62.5% (35/56)              | 78.0% (556/713)            | 18.2% (35/192)     | 96.4% (556/577)    |
|                                                                                       | (48.5-75.1%)               | (74.8-81.0%)               | (13.0-24.4%)       | (94.5-97.7%)       |

\*Compared to result of all three tests (cystoscopy, voided cytology, imaging). Positive=positive on at least one of the three tests; Negative=negative on all three tests.

The following table shows the sensitivity and specificity of voided urine cytology for this study for TCC.

| Voided Cytology: Sensitivity, Specificity, PPV and NPV for Risk factor Patients (T0-T4) |                            |                            |                    |                    |
|-----------------------------------------------------------------------------------------|----------------------------|----------------------------|--------------------|--------------------|
|                                                                                         | Sensitivity (95% Exact CI) | Specificity (95% Exact CI) | PPV (95% Exact CI) | NPV (95% Exact CI) |
| Voided Cytology*                                                                        | 32.8% (15/46)              | 100% (7/13713)             | 100% (15/15)       | 95.8% (7/13714)    |
|                                                                                         | (19.5-48.0%)               | (99.5-100%)                | (78.2-100%)        | (94.1-97.2%)       |

\*Compared to result of all three tests (cystoscopy, voided cytology, imaging). Positive=positive on at least one of the three tests; Negative=negative on all three tests. Not every patient positive for TCC had a cytology result, but every patient negative for TCC did have a cytology result.

The following tables show the NMP22 test results (cut-off  $\geq 7.5$  U/mL) compared to cytology results and the combination of NMP22 results and cytology for the different stages and grades of TCC.

| Percent and Fraction of Positives by stage (T0 <sup>12</sup> ,T4) (95% confidence interval) |                                     |                              |                                    |
|---------------------------------------------------------------------------------------------|-------------------------------------|------------------------------|------------------------------------|
|                                                                                             | NMP22 Level Cut-off $\geq 7.5$ U/mL | Voided Cytology              | NMP22 & Cytology Results Combined* |
| T0 <sup>12</sup>                                                                            | 60.0% (3/5)<br>(14.7-94.7%)         | 0% (0/5)                     | 60.0% (3/5)<br>(14.7-94.7%)        |
| Ta                                                                                          | 45.0% (9/20)<br>(23.1-68.4%)        | 16.3% (3/18)<br>(3.6-41.4%)  | 57.9% (11/19)<br>(33.5-79.8%)      |
| Tb                                                                                          | 80.0% (4/5)<br>(28.4-99.5%)         | 66.7% (2/3)<br>(9.4-99.2%)   | 100% (4/4)<br>(39.8-100%)          |
| T1                                                                                          | 83.3% (7/11)<br>(30.8-99.1%)        | 50.0% (5/10)<br>(18.7-81.3%) | 72.7% (9/11)<br>(39.0-94.0%)       |
| T2, T3, T4                                                                                  | 76.9% (10/13)<br>(46.2-95.0%)       | 55.6% (5/9)<br>(21.1-86.3%)  | 92.3% (12/13)<br>(64.0-99.8%)      |
| Tx                                                                                          | 100% (2/2)<br>(15.8-100%)           | 0% (0/1)<br>(-)              | 100% (2/2)<br>(15.8-100%)          |

\* Positive= positive on either test; Negative= negative on both tests

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| Percent and Fraction of Positives by Grade (95% confidence interval) |                                    |                              |                                      |
|----------------------------------------------------------------------|------------------------------------|------------------------------|--------------------------------------|
|                                                                      | NMP22 Test Cut-off $\geq 7.5$ U/mL | Voided Cytology              | NMP22 and Cytology Results Combined* |
| No malignancy                                                        | 60.0% (3/5)<br>(14.7-94.7%)        | 0% (0/5)<br>(-)              | 60.0% (3/5)<br>(14.7-94.7%)          |
| Low                                                                  | 50.0% (9/18)<br>(26.0-74.0%)       | 13.3% (2/15)<br>(1.7-40.5%)  | 56.3% (11/18)<br>(29.0-80.3%)        |
| Medium                                                               | 70.6% (12/17)<br>(44.0-89.7%)      | 42.9% (6/14)<br>(17.7-71.1%) | 82.4% (14/17)<br>(56.6-96.2%)        |
| High                                                                 | 68.8% (11/16)<br>(41.3-89.0%)      | 58.3% (7/12)<br>(27.7-84.8%) | 87.5% (14/16)<br>(61.7-98.5%)        |

\* Positive= positive on either test; Negative= negative on both tests

## CONCLUSION

Urinary NMP22 test values equal to or greater than 7.5 U/mL in patients with symptoms or risk factors for bladder cancer may indicate the presence of TCC of the bladder. Patients with NMP22 test values below 7.5 U/mL are less likely to have TCC.

| Sensitivity, Specificity, PPV and NPV for Range of Incidence Rates |             |             |       |       |
|--------------------------------------------------------------------|-------------|-------------|-------|-------|
| Incidence Rate                                                     | Sensitivity | Specificity | PPV   | NPV   |
| 1%                                                                 | 62.5%       | 78.0%       | 2.8%  | 99.5% |
| 7.0%                                                               | 62.5%       | 78.0%       | 17.6% | 96.5% |
| 7.3% (actual rate)                                                 | 62.5%       | 78.0%       | 18.2% | 96.4% |
| 15.0%                                                              | 62.5%       | 78.0%       | 33.4% | 92.2% |

Urine NMP22 antigen concentrations should not be interpreted as evidence of the presence or absence of malignant disease in the bladder without corroboration from other diagnostic procedures. Other clinically accepted tests and procedures should be considered in the diagnosis of disease and good patient management.

## Performance Characteristics

### LIMIT OF DETECTION

The lowest concentration of NMP22 antigen that can be measured reliably with the Matritech NMP22 Test Kit is 2.1 U/mL. The minimal detectable level is defined as that NMP22 antigen value which corresponds to the absorbance that is two standard deviations (2SD) above the mean absorbance of twenty replicate determinations of calibrator #1 (0 U/mL).

### PRECISION

Following procedures outlined in the National Committee for Clinical Laboratory Standards (NCCLS) Document EP5-A: Evaluation of Precision Performance of Clinical Chemistry Devices, within-run and total precision were evaluated for three urine controls and five patient specimens. The specimens were assayed in duplicate in each of two independent runs repeated daily over a 20-day period.

| Specimen        | Number | Mean (U/mL) | Within Run %CV | Total %CV |
|-----------------|--------|-------------|----------------|-----------|
| Urine control 1 | 80     | 7.0         | 4.3            | 7.9       |
| Urine control 2 | 80     | 25.8        | 2.5            | 4.9       |
| Urine control 3 | 80     | 51.4        | 2.2            | 3.7       |
| Specimen A      | 80     | 6.3         | 5.0            | 12.4      |
| Specimen B      | 80     | 16.5        | 3.2            | 6.7       |
| Specimen C      | 80     | 31.1        | 2.5            | 5.8       |
| Specimen D      | 80     | 63.0        | 2.3            | 5.4       |
| Specimen E      | 80     | 96.3        | 2.3            | 5.7       |

### RECOVERY

Known concentrations of NMP22 antigen from stabilized patient urine were added to stabilized urine containing low endogenous levels of NMP22 antigen. The samples were measured in duplicate. The mean of 2 assays is reported. Mean recoveries of NMP22 antigen in stabilized urine ranged from 89% to 111% with an overall mean of 99%. An example of a typical recovery study is summarized below:

| NMP22 Antigen Added (U/mL) | NMP22 Antigen Recovered (minus endogenous) | Percent Recovery |
|----------------------------|--------------------------------------------|------------------|
| 48.6                       | 45.9                                       | 94               |
| 31.5                       | 28.4                                       | 90               |
| 23.5                       | 22.3                                       | 95               |
| 11.0                       | 11.4                                       | 103              |
| Mean Recovery              |                                            | 96               |

### LINEARITY OF DILUTION

Six (6) stabilized urine samples containing elevated NMP22 antigen levels were serially diluted with the NMP22 Sample Diluent (NMP22 Urine Calibrator 1 (0 U/mL)) and assayed in triplicate. Linear regression analysis of the NMP22 antigen concentrations versus dilution was performed. The slopes for the 6 samples ranged

from 0.80 to 1.06 with a correlation coefficient of greater than 0.993, thus demonstrating that the samples will dilute linearly.

## Ordering Information and Technical Service

For technical assistance or to place an order contact:  
Inverness Medical  
Phone: 877-441-7440  
Fax: 877-441-7441  
www.invernessmedicalpd.com

### POTENTIALLY INTERFERING SUBSTANCES

Substances listed were evaluated and found to have no significant effects on the results of the Matritech NMP22 Test Kit at the following concentrations:

| Substance                     | Concentration          |
|-------------------------------|------------------------|
| <b>Urine Analytes</b>         |                        |
| Protein (Human serum albumin) | 100 mg/dL              |
| Protein (Human IgG)           | 100 mg/dL              |
| Hemoglobin                    | 1.6 mg/dL              |
| Red Blood Cells (#/dL)        | 1.8 x 10 <sup>11</sup> |
| Whole Blood (v/v%)            | 1.0 %                  |
| Glucose                       | 20.0 mg/dL             |
| Cyokeratins (TPA )(U/dL)      | 40 U/dL                |

|                           |                       |
|---------------------------|-----------------------|
| <b>Therapeutic Agents</b> |                       |
| BCG (live)(CFU/mL)        | 5.0 x 10 <sup>6</sup> |
| Thiotepa                  | 60.0 mg/dL            |

### Dugs

|                         |            |
|-------------------------|------------|
| Digoxin                 | 0.05 mg/dL |
| Acetaminophen           | 20 mg/dL   |
| Sodium Ascorbate        | 20 mg/dL   |
| Caffeine                | 20 mg/dL   |
| Sodium Salicylate       | 20 mg/dL   |
| Sodium Acetylsalicylate | 20 mg/dL   |
| Ampicillin              | 20 mg/dL   |
| Tetracycline            | 20 mg/dL   |

## Limitations

- NMP22 antigen concentrations should not be interpreted as evidence of the presence or absence of malignant disease in the bladder without corroboration from other diagnostic procedures and should only be used in conjunction with other diagnostic information in the management or diagnosis of patients with transitional cell carcinoma of the bladder.
- Patients with known malignancy of the bladder or transitional cell carcinoma in other parts of the urinary tract may have urinary NMP22 antigen levels within the range of concentrations observed in individuals with no known malignancy.
- For increased ability to accurately detect the NMP22 antigen, clinical studies investigating the use of the NMP22 test in the management of post-surgical TCC patients have shown that the urine sample must be collected between the hours of midnight and noon (0:00 to 12:00 hours)<sup>19</sup>. The clinical trial investigating the use of the NMP22 test in the initial diagnosis of patients with TCC did not restrict the collection time. For details on urine collection refer to NMP22 Urine Collection Kit Instructions for Use.
- Elevated urinary NMP22 antigen levels have been observed in individuals with no known malignancy of the urinary tract. Occasional elevations have been observed immediately after extreme exercise (e.g. running more than 10 miles) in apparently healthy individuals, in some benign conditions (e.g. interstitial cystitis, urinary tract infections), in patients with renal cancer and malignancy of any site undergoing systemic chemotherapy. Elevated values are always seen in patients who have undergone total cystectomy. Significance of these elevated results is unknown. Physicians should use some judgement in determining when samples are collected.
- Samples collected fewer than 5 days after an invasive procedure such as cystoscopy or catheterization of the urethra may result in elevated values due to tissue damage.
- Samples collected while the patient is undergoing intravesical therapy may not accurately reflect the presence or absence of malignancy in the bladder. Interpretation of NMP22 test results from these samples has not been adequately determined.
- Only urine that has been stabilized with the NMP22 Urine Stabilizer should be used in this assay. Unstabilized urine and other body fluids should not be used in this test.



## Product Information

NMP22® Test Kit  
Manufactured by Binax Inc.  
Scarborough, ME 04074 USA  
Phone: 877-441-7440  
Fax: 877-441-7441  
Catalog Number: D1100  
96 Determinations

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Protected by U.S. Patent Nos. 4,882,268; etc. and RE35, 474; other rights granted and pending.

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

## Intended Use

The Matritech NMP22 Test Kit is an enzyme immunoassay (EIA) for the *in vitro* quantitative determination of the nuclear mitotic apparatus protein (NuMA) in stabilized voided urine. The Matritech NMP22 Test Kit is indicated as an aid 1) in the diagnosis of persons with symptoms or risk factors for transitional cell cancer (TCC) of the bladder (cut-off  $\geq 7.5$  U/mL) in conjunction with, and not in lieu of, current standard diagnostic procedures, and 2) in the management of patients with transitional cell carcinoma of the bladder, after surgical treatment to identify those patients with occult or rapidly recurring TCC (cut-off  $> 10$  U/mL).

## Contraindications, Warnings and Precautions

There are no known contraindications for the Matritech NMP22 Test Kit.

### PRECAUTIONS

- For *In vitro* diagnostic use.
- Specimens used in this kit must be collected following the Matritech NMP22 Urine Collection Kit instructions and stabilized with the NMP22 Stabilizer according to Collection Kit instructions for use.
- Do not mix components from different kit lots.
- Do not use components after the expiration date.
- Use only plastic containers to process or store urine samples, calibrators, sample diluent or controls. Do not use any glass containers other than containers in which calibrators and controls are supplied.
- Refer to local regulations for the disposal of medical waste when disposing of any remaining kit reagents or specimens.
- Store the Matritech NMP22 Urine Collection Kit at a controlled room temperature of 50 to 70°F (10 to 25°C).
- Do not use the Matritech NMP22 Urine Collection Kit beyond the expiration date.

### WARNINGS

- Handle all specimens as if capable of transmitting infection.
- Do not eat, drink, smoke or pipette by mouth where kit reagents are being handled.
- Wear protective laboratory devices such as gloves and laboratory coats when handling specimens and kit reagents; wash hands afterwards.
- The calibrators, controls and sample diluent contain human albumin. Each lot of human albumin is tested and found to be negative for antibody to HIV-1, HIV-2, HbsAg and HCV by FDA approved tests. Because no test method can offer complete assurance that these reagents do not contain HIV, Hepatitis or other infectious agents, reagents should be handled at the BSL 2 as recommended for any potentially infectious human serum or blood specimen. [see CDC-NIH manual; Biosafety in Microbiological and Biomedical Laboratories, 1984 page 12-16].
- OPD tablets and Sulfuric Acid Stop Solution are known skin and mucous membrane irritants.
- The stabilizer found in the Urine Collection Container of the Matritech NMP22 Urine Collection Kit and the calibrators, controls and sample diluent found in the NMP22 Test Kit contain trace amounts of Fungizone™ and gentamicin as antimicrobial agents, which may be toxic if ingested.

For *in vitro* diagnostic use.

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## Instructions for Use

### REAGENTS REQUIRED BUT NOT PROVIDED

- Matritech NMP22 Urine Collection Kit, catalogue number D2000
- 2 Molar (4 Normal) Sulfuric Acid Stop Solution; see Assay Preparation

### MATERIALS NOT PROVIDED

- 200  $\mu$ L precision pipette with disposable tips
- Precision pipette with disposable tips to deliver 2 mL
- microplate reader (490 nm)
- microplate washer/aspiration device capable of delivering at least 310  $\mu$ L per well of solution with a 10 second soak between aspirations (optional)
- centrifuge
- plastic test tubes to prepare OPD solution
- plastic test tubes to prepare specimens (centrifuging, or diluting specimens)

### Storage Instructions

- Store kit components at 2-8°C.
- Components as packaged are stable through the expiration date printed on the kit box label.
- Bring all reagents to room temperature (18-25°C) prior to use.
- Immediately after use, store all reagents at 2-8°C except the diluted wash solution which should be kept at room temperature.
- Reconstituted calibrators, controls and sample diluent are stable for 14 days at 2-8°C or for 30 days when stored at -20°C or lower in single-use aliquots.
- Do not allow aliquots to experience more than one freeze-thaw.
- Keep foil packaging for OPD tablets sealed until ready to use.

### Specimen Collection

#### PATIENT PREPARATION

Persons should not be tested with the Matritech NMP22 Test if they meet any of the following criteria:

- Have had a total cystectomy
- Less than five (5) days after an invasive procedure, such as cystoscopy or catheterization

#### SPECIMEN COLLECTION

A single void of urine should be collected and **STABILIZED IMMEDIATELY** by the patient or medical personnel, using the Matritech NMP22 Urine Collection Kit, catalogue number D2000. Collection should be done between midnight and noon (0:00 to 12:00 hours) if it is for post-surgical patient management. DO NOT USE OTHER METHODS FOR COLLECTING URINE SAMPLES. INVALID NMP22 ANTIGEN MEASUREMENTS WILL RESULT FROM IMPROPERLY COLLECTED SAMPLES OR SAMPLES THAT ARE NOT PROPERLY STABILIZED.

- Stabilized urine collected with the Matritech NMP22 Urine Collection Kit should be blue/green in color.
- Stabilized samples may remain at room temperature (18-25°C) for up to 96 hours after collection before being processed (see SAMPLE PREPARATION for storage times after processing). After 96 hours they must be stored at 2-8°C. **If shipping temperatures are expected to exceed 35°C, samples should be processed and shipped FROZEN on dry ice.**
- Sodium azide or other preservatives should not be added to the samples as incorrect results may be obtained when analyzed with the Matritech NMP22 Test Kit.

#### SAMPLE PREPARATION

- Process the stabilized urine specimen as follows:
  - Ensure that each specimen is at room temperature (18-25°C) prior to processing.
  - Centrifuge the total contents of the specimen in plastic tubes at 500 to 1000 x G for 10 to 15 minutes at 10 to 25°C to remove precipitates.
  - Decant the supernatant into a separate plastic container.
- Analyze each sample supernatant using the Matritech NMP22 Test Kit procedure.
- Samples that have been centrifuged and decanted [processed] may be stored at 2-8°C for up to 1 week prior to measurement. Alternatively, samples may be stored at -20°C or lower. -20°C sample storage should be limited to 8 weeks as antigen loss has been reported in some samples after 8 weeks. For storage longer than 8 weeks, -80°C is required. Samples should be thawed at room temperature. Thawing at elevated temperatures (greater than 25°C) may result in loss of antigen activity. Avoid more than three freeze/thaws.
- Stored samples should be centrifuged and decanted a second time to remove any additional precipitates before testing.

### Procedural Notes

- It is important that the user be familiar with the procedure.
- A standard curve must be established with every run.
- Disposable pipette tips should be used to prevent cross-specimen or cross-reagent contamination.
- Avoid interference caused by detergents or other contaminants of labware by thoroughly rinsing prior to use.
- Adherence to protocol incubation times and temperatures is necessary to achieve valid results.

- Avoid microbial contamination of reagents when removing aliquots from the vials. Microbial contamination should be suspected if the reagent solutions become cloudy or emit strong odor.
- Do not expose OPD reagents to light, any oxidizing agents or metal during storage or incubation.
- Acid Stop Solution is 2 Molar [4 Normal] Sulfuric Acid. Any other concentration of acid will produce adverse results. This acid may be purchased commercially or prepared from concentrated acid. See ASSAY PREPARATION.
- Do not place samples of urine, controls or calibrators in glass containers other than the containers in which the reagents were supplied.
- Do not allow the microplate to become dry during any part of the assay procedure.

### Quality Control

Good laboratory practices include the use of control specimens within each assay to ensure that all reagents and procedures are performed properly. The Matritech NMP22 Test Kit contains a set of tri-level controls, which can be used to verify assay performance. Suggested concentration ranges for each control level are printed on the vial labels. Because each laboratory may obtain slightly different results, it is suggested that each laboratory establish its own range for each level of urine control.

### Procedure

#### ASSAY PREPARATION

- Allow kit components and processed stabilized urine samples to equilibrate to room temperature (18-25°C) for 15 to 20 minutes before use.
- Gently swirl all reagents to ensure mixing prior to use.
- Verify the samples have been prepared as outlined in SAMPLE PREPARATION.
- Prepare Wash Solution from the 100 X Wash Concentrate. Allow 100X Wash Concentrate to reach room temperature (18-25°C). If any precipitate is present, warm the concentrate until the precipitate dissolves.
- Dilute the 100X Wash Concentrate 1:100 with deionized water. NOTE: 10 mL of concentrate makes 1000mL of wash solution. The vial contains 30 mL.
- Prepare 2M (4 N) Sulfuric Acid Stop Solution by adding 11.1 mL of concentrated sulfuric acid (18 Molar = 36 Normal) to a final volume of 100 mL in deionized water. Store at room temperature.
- Reconstitute each Calibrator and Control with 2 mL deionized water. Reconstitute the Sample Diluent with 10 mL deionized water. Recap and invert stand at room temperature (18-25°C) for approximately 10 minutes. Invert and swirl gently, do not vortex. Let stand an additional 10 minutes before use. Be sure the lyophilized material is completely dissolved before use.
- Coated Microplate Strip Well foil package should be opened by cutting close to the package edge with the zip-lock strip. Remove strip wells not required from the plate frame. Return all unused, coated strip wells to the foil package along with the desiccant packet. Carefully reseal the foil package with the zip-lock closure and store at 2-8°C.

#### ASSAY PROCEDURE

- Wash NMP22 antibody Coated Strip Well Plate 3 times with the Wash Solution using either an automatic microplate wash aspiration system or manually. For the automatic washing system, settings should allow each well to fill completely (minimum 310  $\mu$ L) with a 10-second soak prior to aspiration. After the three cycles are completed the wells should be free of residual wash solution. If necessary, tap the plate on absorbent paper.  
*Proceed immediately to step 2.*
- Pipet 200  $\mu$ L/well of each calibrator, control and sample into assigned duplicate wells. Incubate 2 hours  $\pm$  5 minutes at 18-25°C.
- Wash plate 3 times with Wash Solution as described in step 1.
- Pipet 200  $\mu$ L/well of Digoxigenin Anti-NMP22 Reagent (DIG--22) to all assay wells. Incubate 1 hour  $\pm$  2 minutes at 18-25°C.
- Wash plate 3 times with Wash Solution as described in step 1.
- Pipet 200  $\mu$ L/well of HRP-SAD Reagent to all assay wells. Incubate 30 minutes  $\pm$  2 minutes at 18-25°C.  
NOTE: During the HRP-SAD Reagent incubation, prepare the OPD Solution by transferring the required amount of Color Development Buffer (10 mL for half plate, 20 mL for whole plate) into a plastic test tube. Add one OPD tablet/10 mL of Development Buffer. Cover tube with foil and store in darkened area until ready to use. *Prepare this solution no more than 30 minutes before use.* The tablet(s) should be completely dissolved before use. Avoid contact of metal objects with this solution.
- Wash plate 3 times with Wash Solution as described in step 1.
- Pipet 200  $\mu$ L/well of OPD Solution (mix well before use) to each assay well. Incubate 30 minutes  $\pm$  2 minutes at 18-25°C. *Place developing plate in a darkened area during color development.*
- Stop the color development reaction by adding 50 $\mu$ L/well of 2M(4N) H<sub>2</sub>SO<sub>4</sub> Stop Solution. Tap the plate gently to mix the acid with OPD. Place plate in darkened area at 18-25°C.
- Read the reaction within 10 - 30 minutes of the addition of the Acid Stop Solution using a plate reading spectrophotometer that has been set at 490nm and blanked to zero absorbance with deionized water. NOTE: To eliminate the possibility of reading errors, the microtiter well used to blank the instrument should only contain deionized water.

## Results

### COMPUTER ASSISTED METHOD

Computer assisted data reduction may be used to calculate results. The performance data in this insert were calculated using a point-to-point curve fit. Curve fitting based on a linear regression analysis has also been validated. It is recommended that each laboratory selects either a point-to-point or linear regression curve fit method. Curve fitting based upon other non-linear analysis methods such as log-logit or 4-parameter logistic fit is not recommended. The same curve fit method should be used to calculate results from different assay runs to ensure consistent values are obtained.

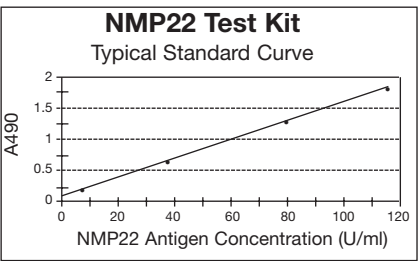
### MANUAL METHOD

The standard curve may be constructed manually on rectilinear graph paper by plotting the mean A490 absorbance value for each set of calibrator replicates on the y-axis versus concentration in U/mL on the x-axis. The best curve fit (straight line) should be drawn through the data points. To determine the NMP22 antigen concentration in a patient sample, find the point on the curve corresponding to the mean absorbance at 490nm of the patient sample and drop a vertical line to the x-axis. Read the concentration of NMP22 antigen in units per milliliter (U/mL).

### ELEVATED SAMPLE RESULTS

If the sample has a mean absorbance at 490nm greater than the mean absorbance of Calibrator #5, the sample should be diluted and re-run. Dilute the sample with the Sample Diluent so that the value obtained will fall within the range of the calibrator curve. When calculating the actual value of the diluted sample, the NMP22 antigen value obtained in the assay should be multiplied by the dilution factor to obtain the actual NMP22 antigen concentration. For example, if a sample is diluted 1 part sample to 1 part sample diluent (1 part sample in 2 parts total volume) the dilution factor is 2. If the diluted sample NMP22 antigen value is 80U/mL, the reported value should be 160 U/mL (80 U/mL X 2).

### EXAMPLE OF STANDARD CURVE



| Calibrator | Rep 1 | Rep 2 | Mean  | %CV |
|------------|-------|-------|-------|-----|
| 0          | 0.079 | 0.080 | 0.080 | 0.9 |
| 7.5        | 0.202 | 0.201 | 0.202 | 0.4 |
| 36         | 0.636 | 0.629 | 0.632 | 0.8 |
| 76         | 1.253 | 1.259 | 1.256 | 0.3 |
| 112        | 1.782 | 1.803 | 1.792 | 0.8 |

| Samples     | Rep 1 | Rep 2 | Mean  | %CV | Rep 1 | Rep 2 | Mean |
|-------------|-------|-------|-------|-----|-------|-------|------|
| Control I   | 0.200 | 0.190 | 0.195 | 3.6 | 7.7   | 7.1   | 7.4  |
| Control II  | 0.441 | 0.433 | 0.437 | 1.3 | 23.4  | 22.8  | 23.1 |
| Control III | 0.797 | 0.763 | 0.780 | 3.1 | 46.4  | 44.2  | 45.3 |
| Sample 1    | 0.122 | 0.122 | 0.122 | 0.0 | 2.7   | 2.7   | 2.7  |
| Sample 2    | 0.417 | 0.421 | 0.419 | 0.7 | 21.8  | 22.1  | 21.9 |
| Sample 3    | 1.328 | 1.382 | 1.355 | 2.8 | 80.8  | 84.3  | 82.6 |

### ACCEPTANCE OF RESULTS

#### Calibrators:

The high calibrator (nominal 120 U/mL) should have an absorbance between 0.9 – 2.4.

The lowest calibrator (0 U/mL) should have an absorbance less than 0.15.

#### Tri-Level Controls:

- Recovery of control concentrations should fall within established ranges.

### REPORTING OF RESULTS

NMP22 antigen values within the range of the standard curve may be reported to physicians. If NMP22 antigen values are found to be greater than the high calibrator, it is recommended that the sample be diluted using NMP22 antigen sample diluent and re-assayed. The re-assayed value, corrected for its dilution, should be reported.

Values of  $\geq$ 7.5 U/mL were found to be optimal for identifying patients with a first occurrence of bladder cancer in the clinical trial reported within this insert. Values of  $>$ 10 U/mL were found to be optimal for identifying patients at risk for occult or rapidly recurring bladder cancer in the clinical trial reported within this insert.

## Expected Values

### Management of TCC of the Bladder (cut-off $>$ 10.0 U/mL)

A prospective clinical trial was performed at 14 institutions and Matritech to determine the utility of the NMP22 Test in identifying patients at risk for occult or rapidly recurring transitional cell carcinoma of the bladder (TCC) <sup>11</sup>. A total of 706 subjects were enrolled: 398 normal healthy volunteers, 117 subjects with benign urological diseases, 98 subjects with malignancies other than TCC, and 93 subjects with TCC who experienced at least 1 disease episode while enrolled in the trial. A disease episode is defined as the following: 1) performance of a surgical procedure for primary or recurring TCC, such as biopsy, fulguration, or transurethral resection of a tumor, or partial cystectomy or unilateral ureteronephrectomy; 2) collection of a urine sample according to the method described in the Matritech NMP22 Urine Collection Kit instructions, between 5 and 60 days after surgical procedure; and 3) performance of a procedure allowing assessment of presence of a neoplasm in bladder, urethra, ureters or pelvis of the kidney, such as cystoscopic examination or total cystectomy, between 2 and 6 months after the surgical procedure. A total of 128 disease episodes occurred among 93 subjects in the trial. To determine sensitivity and specificity, the disease episodes were classified as negative (no lesions seen on cystoscopy, or if a lesion was seen, pathologic examination of tissue indicated no abnormality present, or atypia or dysplasia), positive (pathologic examination of tissue indicated malignancy present), or unknown (lesion seen but no tissue collected for pathologic examination).

Samples were collected from normal healthy volunteers when each subject had no symptoms of a urologic abnormality and was not under a physician's care for a urologic condition, from subjects with benign urologic conditions when the subject was receiving treatment from a physician for that condition, and from subjects with cancers other than TCC when the subject was being treated or followed for that malignancy by a physician.

The percent distribution of NMP22 antigen levels in self-referred healthy subjects, patients under treatment for benign diseases, and patients with malignancies of other sites is presented in the following table. The malignancy group includes those with active disease under treatment and those with malignancy diagnosed within the previous 18 months but which was no longer clinically evident.

| Percent Distribution of NMP22 Antigen(U/mL) |        |       |              |           |            |         |
|---------------------------------------------|--------|-------|--------------|-----------|------------|---------|
|                                             | Number | 0-10  | $\geq$ 10-20 | $>$ 20-50 | $>$ 50-100 | $>$ 100 |
| <b>Healthy Subject</b>                      |        |       |              |           |            |         |
| Male $\geq$ 50 years                        | 215    | 94.9% | 3.3%         | 1.9%      | 0%         | 0%      |
| Female $\geq$ 50 years                      | 151    | 87.3% | 6.6%         | 4.6%      | 0.7%       | 0.7%    |
| $<$ 50 years both sexes                     | 32     | 90.6% | 9.4%         | 0%        | 0%         | 0%      |
| <b>Total</b>                                | 398    | 91.7% | 5.0%         | 2.8%      | 0.3%       | 0.3%    |
| <b>Benign Disease*</b>                      |        |       |              |           |            |         |
| UTI and Cystitis                            | 26     | 84.6% | 11.5%        | 3.8%      | 0%         | 0%      |
| Urinary Calculi                             | 16     | 93.8% | 0%           | 0%        | 6.3%       | 0%      |
| BPH & Prostatitis                           | 52     | 92.3% | 7.7%         | 0%        | 0%         | 0%      |
| Other                                       | 37     | 83.8% | 5.4%         | 8.1%      | 2.7%       | 0%      |
| <b>Total</b>                                | 117    | 88.0% | 7.7%         | 3.4%      | 0.9%       | 0%      |
| <b>Cancers other than TCC</b>               |        |       |              |           |            |         |
| Head and Neck                               | 6      | 83.3% | 0%           | 16.7%     | 0%         | 0%      |
| GI Tract                                    | 12     | 83.3% | 0%           | 8.3%      | 0%         | 8.3%    |
| Cardiovascular & Pulmonary                  | 12     | 58.3% | 8.3%         | 16.7%     | 16.7%      | 0%      |
| Leukemia/Lymphoma                           | 11     | 63.6% | 18.2%        | 9.1%      | 9.1%       | 0%      |
| Prostate                                    | 22     | 81.8% | 0%           | 13.6%     | 4.5%       | 0%      |
| Kidney (non-TCC)                            | 1      | 77.8% | 11.1%        | 11.1%     | 0%         | 0%      |
| Other**                                     | 17     | 64.7% | 17.6%        | 5.9%      | 0%         | 11.8%   |
| <b>Total</b>                                | 98     | 73.5% | 10.2%        | 9.2%      | 4.1%       | 3.1%    |

\*Some patients are included in more than one category

\*\*The "other" category in the cancers other than TCC included malignancies of the bones, joints, cartilage, breast, uterine cervix, other female organs, testes and thyroid, pheochromocytoma, and hemangiopericytoma of the leg.

In this study 92% of the healthy subjects had NMP22 antigen concentrations of 10.0 U/mL or lower. Each laboratory should establish its own reference value.

Of the total 128 disease episodes (See EXPECTED VALUE for description of a disease episode) 116 could be classified as negative (no evidence of malignancy) or positive (occult or rapidly recurring malignant disease present). The first disease episode for each TCC subject in the trial was also analyzed; among 93 subjects, 87 had first disease episodes that could be classified as negative or positive. The following tables show the NMP22 Test results relative to a reference value of  $>$ 10.0 U/mL for patients with occult or rapidly recurring TCC, following surgical treatment for TCC, and for patients with no malignant disease present.

These tables and analyses are broken down into the following categories: (1) all episodes of disease (multiple events per patient), (2) first episode of disease (3) sexes combined, (4) sexes separately, due to differences in NMP22 Test values seen between the sexes.

| Sexes Combined; All Disease Episodes                      |                                                    |                                  |       |
|-----------------------------------------------------------|----------------------------------------------------|----------------------------------|-------|
| (cystoscopic exam 2 to 6 months after Surgical Treatment) |                                                    |                                  |       |
| NMP22 (U/mL) Value                                        | Occult/Rapidly Recurring Malignant Disease Present | No Evidence of Malignant Disease | Total |
| $\leq$ 10 U/mL                                            | 10                                                 | 64                               | 74    |
| $>$ 10 U/mL                                               | 24                                                 | 18                               | 42    |
| <b>Total</b>                                              | 34                                                 | 82                               | 116   |
|                                                           |                                                    | 95% confidence level             |       |
| Sensitivity                                               | 70.6 %                                             | 55.3 - 85.9                      |       |
| Specificity                                               | 78.0%                                              | 69.0 - 87.0                      |       |
| Accuracy                                                  | 75.9%                                              | 68.1 - 83.7                      |       |
| Positive predictive value                                 | 57.1%                                              | 42.1 - 72.1                      |       |
| Negative predictive value                                 | 86.5%                                              | 78.7 - 94.3                      |       |

| Sexes Combined; First Disease Episodes                    |                                                    |                                  |       |
|-----------------------------------------------------------|----------------------------------------------------|----------------------------------|-------|
| (cystoscopic exam 2 to 6 months after Surgical Treatment) |                                                    |                                  |       |
| NMP22 (U/mL) Value                                        | Occult/Rapidly Recurring Malignant Disease Present | No Evidence of Malignant Disease | Total |
| $\leq$ 10 U/mL                                            | 6                                                  | 46                               | 52    |
| $>$ 10 U/mL                                               | 19                                                 | 16                               | 35    |
| <b>Total</b>                                              | 25                                                 | 62                               | 87    |
|                                                           |                                                    | 95% confidence level             |       |
| Sensitivity                                               | 76.0%                                              | 59.3 - 92.7                      |       |
| Specificity                                               | 74.2%                                              | 63.3 - 85.1                      |       |
| Accuracy                                                  | 74.7%                                              | 65.6 - 83.8                      |       |
| Positive predictive value                                 | 54.3%                                              | 37.8 - 70.8                      |       |
| Negative predictive value                                 | 88.5%                                              | 79.8 - 97.2                      |       |

| Males; All Disease Episodes                               |                                                    |                                  |       |
|-----------------------------------------------------------|----------------------------------------------------|----------------------------------|-------|
| (cystoscopic exam 2 to 6 months after Surgical Treatment) |                                                    |                                  |       |
| NMP22 (U/mL) Value                                        | Occult/Rapidly Recurring Malignant Disease Present | No Evidence of Malignant Disease | Total |
| $\leq$ 10 U/mL                                            | 8                                                  | 58                               | 66    |
| $>$ 10 U/mL                                               | 19                                                 | 11                               | 30    |
| <b>Total</b>                                              | 27                                                 | 69                               | 96    |
|                                                           |                                                    | 95% confidence level             |       |
| Sensitivity                                               | 70.4%                                              | 53.2 - 87.6                      |       |
| Specificity                                               | 84.1%                                              | 75.5 - 92.7                      |       |
| Accuracy                                                  | 80.2%                                              | 72.2 - 88.2                      |       |
| Positive predictive value                                 | 63.3%                                              | 46.1 - 80.5                      |       |
| Negative predictive value                                 | 87.9%                                              | 80.0 - 95.8                      |       |

| Males; First Disease Episodes                             |                                                    |                                  |       |
|-----------------------------------------------------------|----------------------------------------------------|----------------------------------|-------|
| (cystoscopic exam 2 to 6 months after Surgical Treatment) |                                                    |                                  |       |
| NMP22 (U/mL) Value                                        | Occult/Rapidly Recurring Malignant Disease Present | No Evidence of Malignant Disease | Total |
| $\leq$ 10 U/mL                                            | 4                                                  | 43                               | 47    |
| $>$ 10 U/mL                                               | 16                                                 | 11                               | 27    |
| <b>Total</b>                                              | 20                                                 | 54                               | 74    |
|                                                           |                                                    | 95% confidence level             |       |
| Sensitivity                                               | 80.0%                                              | 62.5 - 97.5                      |       |
| Specificity                                               | 79.6%                                              | 68.9 - 90.3                      |       |
| Accuracy                                                  | 79.7%                                              | 70.5 - 88.9                      |       |
| Positive predictive value                                 | 59.3%                                              | 40.8 - 77.8                      |       |
| Negative predictive value                                 | 91.5%                                              | 83.5 - 99.5                      |       |

| Females; All Disease Episodes                             |                                                    |                                  |       |
|-----------------------------------------------------------|----------------------------------------------------|----------------------------------|-------|
| (cystoscopic exam 2 to 6 months after Surgical Treatment) |                                                    |                                  |       |
| NMP22 (U/mL) Value                                        | Occult/Rapidly Recurring Malignant Disease Present | No Evidence of Malignant Disease | Total |
| $\leq$ 10 U/mL                                            | 2                                                  | 6                                | 8     |
| $>$ 10 U/mL                                               | 5                                                  | 7                                | 12    |
| <b>Total</b>                                              | 7                                                  | 13                               | 20    |
|                                                           |                                                    | 95% confidence level             |       |
| Sensitivity                                               | 71.4%                                              | 37.9 - 100                       |       |
| Specificity                                               | 46.2%                                              | 19.1 - 73.3                      |       |
| Accuracy                                                  | 55.0%                                              | 33.2 - 76.8                      |       |
| Positive predictive value                                 | 41.7%                                              | 13.8 - 69.6                      |       |
| Negative predictive value                                 | 75.0%                                              | 45.0 - 100                       |       |

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| Females; First Disease Episodes                           |                                                    |                                  |       |
|-----------------------------------------------------------|----------------------------------------------------|----------------------------------|-------|
| (cystoscopic exam 2 to 6 months after Surgical Treatment) |                                                    |                                  |       |
| NMP22 (U/mL) Value                                        | Occult/Rapidly Recurring Malignant Disease Present | No Evidence of Malignant Disease | Total |
| $\leq$ 10 U/mL                                            | 2                                                  | 3                                | 5     |
| $>$ 10 U/mL                                               | 3                                                  | 5                                | 8     |
| <b>Total</b>                                              | 5                                                  | 8                                | 13    |
|                                                           |                                                    | 95% confidence level             |       |
| Sensitivity                                               | 60.0%                                              | 17.1 - 100                       |       |
| Specificity                                               | 37.5%                                              | 4.0 - 71.0                       |       |
| Accuracy                                                  | 46.2%                                              | 19.1 - 73.3                      |       |
| Positive predictive value                                 | 37.5%                                              | 4.0 - 71.0                       |       |
| Negative predictive value                                 | 60.0%                                              | 17.1 - 100                       |       |

As indicated by the above statistical analysis, urinary NMP22 test values of greater than 10.0 U/mL for samples collected following a surgical procedure may indicate occult or rapidly recurring malignant disease of the bladder. Patients with NMP22 test values equal to or below 10.0 U/mL are less likely to have malignant disease on follow-up two to six months later.

| Sensitivity, Specificity, PPV and NPV for Management of patients by Range of Incidence Rates |             |             |       |       |  |
|----------------------------------------------------------------------------------------------|-------------|-------------|-------|-------|--|
| Incidence Rate                                                                               | Sensitivity | Specificity | PPV   | NPV   |  |
| 10%                                                                                          | 76.0%       | 74.2%       | 24.7% | 96.5% |  |
| 20%                                                                                          | 76.0%       | 74.2%       | 42.4% | 92.5% |  |
| 30%                                                                                          | 76.0%       | 74.2%       | 55.8% | 87.8% |  |

### Diagnosis of TCC of the Bladder (cut-off $\geq$ 7.5 U/mL)

A second prospective clinical trial was performed at 33 sites to determine the utility of the NMP22 test as an aid in diagnosing transitional cell carcinoma of the bladder (TCC). Voided urine samples were collected from a total of 1147 individuals: 769 patients with unresolved hematuria or other symptoms or risk factors for bladder cancer (e.g. dysuria, exposure to carcinogens, history of smoking), 329 self-referred, hematuria negative, normal healthy volunteers, and 49 patients with active cancers other than those of the urinary tract.

To determine sensitivity and specificity, patients with risk factors for bladder cancer were classified as positive or negative for TCC. Patients were considered negative for TCC if their evaluation included a negative voided cytology, cystoscopy and upper tract evaluation (such as IVP or ultrasound). A negative voided cytology was defined as one in which no malignant or dysplastic cells were identified. A result of suspicious cells required further evaluation until the diagnosing physician deemed that no further diagnostic procedures were necessary at that time. Negative cystoscopy and upper tract evaluations were defined as those in which no tumor was identified, or if identified, was pathologically confirmed as non-malignant. Patients were considered positive for TCC if they had a positive cytology and/or cystoscopy and/or upper tract diagnostic procedure. A positive cytology was defined as one in which malignant or dysplastic cells were present. A result of suspicious cells required further evaluation. A positive cystoscopy was defined as one in which a tumor was seen endoscopically, and for which there was pathologic confirmation of TCC of biopsied or resected tissue. A positive upper tract evaluation was defined as one in which a tumor, filling defect, or wall thickening was identified and there was pathologic confirmation of malignancy of biopsied or resected tissue. No patients were found positive for upper tract cancer.

A single voided urine sample was collected from each patient with symptoms or risk factors for bladder cancer during their standard diagnostic evaluation. Samples from normal healthy volunteers were collected when each subject had no symptoms of a urologic abnormality, and had no history of a urologic disease during the prior twelve months. Samples were tested for hematuria by dipstick to rule out undiagnosed disease. Only samples that were negative for blood were included in the analysis. Samples from patients with other cancers were collected when the patients had clinically or pathologically confirmed malignancy and were not undergoing chemo-, immuno- or radiation therapy at the time of collection. These patients must not have been diagnosed with a urinary tract disease within the prior twelve months.

The percent distribution of NMP22 antigen levels in hematuria-negative healthy subjects, persons with risk factors for TCC newly diagnosed with benign disease (as yet untreated), and persons diagnosed with cancers other than the bladder and not yet receiving treatment, is presented in the following table.

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