

SUMMARY OF SAFETY AND EFFECTIVENESS DATA

I. General Information

Device Generic Name: Tumor Associated Antigen Immunoassay System

Device Trade Name: Matritech NMP22 Test Kit

Applicant's Name and Address: Matritech, Inc.
330 Nevada Street
Newton, MA 02160

Premarket Approval Application (PMA) Number: P940035, Supplement 2

Date of Panel Recommendations: December 13, 1999

Date of Notice of Approval of Application

JAN 18 2000

II. Indications for Use

Intended Use

The Matritech NMP22 Test Kit is an enzyme immunoassay (EIA) for the in vitro quantitative determination of nuclear matrix protein NMP22 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 ≥ 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 NMP22 Test Kit.

- Elevated urinary NMP22 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 results from these samples has not been adequately determined.

See attached labeling for additional warnings and precautions.

III. Device Description

Nuclear matrix proteins (NMP) make up the internal structural framework of the nucleus^{1,2} and are associated with such functions as DNA replication, RNA synthesis, and hormone binding.^{3,4,5} Further work has indicated that NMPs are involved in regulation and coordination of gene expression.^{4,6,7} Later work by Fey and Penman⁸ demonstrated that NMP expression varied with cell type of origin. This observation was followed by work showing that soluble NMPs could be detected in the serum of cancer patients in higher concentrations than were found in normal serum.⁹ Partin and colleagues¹⁰ demonstrated that the pattern of expression of NMP differed in normal prostate tissue, benign prostatic hyperplasia, and prostate cancer. Previous work has identified specific NMPs to osteosarcoma¹¹, colon¹² and breast cancer¹³. These observations indicated that measurement of NMP may have clinical utility in the management of a number of malignancies.

The antibodies contained within this assay recognize the head and rod domains of NuMA, nuclear mitotic apparatus protein. NuMA has been shown to be present in malignant tissues at levels more than ten times higher than in normal tissues. The NuMA antigen moiety detected by the Matritech NMP22 Test Kit is referred to as NMP22. In the urine of healthy individuals, NMP22 is present at low levels. The majority of patients with bladder cancer have been shown to release large quantities of NMP22 into the urine. The assay is designed to quantify NMP22 in stabilized voided urine. Patients with TCC/UT present in the urinary tract have been shown to release higher levels of NMP22 into the urine. This assay is designed to quantify NMP22 in stabilized voided urine.

The Matritech NMP22 Test Kit is an enzyme immunoassay in a 96 well microtiter strip-well format. The assay employs two murine monoclonal antibodies that are specific for the nuclear matrix protein NMP22.

Calibrators, controls, or stabilized patient urine react with an antibody coated onto wells of a microtiter plate. After washing, the captured NMP22 antigen reacts with a second antibody labeled with digoxigenin. After a wash, the digoxigenin-labeled antibody is detected with an anti-digoxigenin antibody labeled with horseradish peroxidase using o-phenylenediamine substrate.

The reaction is terminated by the addition of 2 molar sulfuric acid. The concentration of antigen in the urine is proportional to the intensity of color development and the actual concentration of NMP22 is determined from a standard curve. The standard curve is constructed from the concurrent testing of the NMP22 Urine Calibrators which range from 0 to approximately 120 U/mL.

IV. Alternative Practices and Procedures

The Matritech NMP22 Test Kit is used in conjunction with conventional medical practices and procedures employed to diagnose patients with carcinoma of the urinary tract. Current methods for diagnosis and monitoring of tumors of the bladder and urethra include cystoscopic examination and cytopathologic examination of cells in voided urine or bladder washings. Current methods for diagnosis and monitoring of tumors in the ureters or renal pelvis include endoscopic examination and intravenous pyelography. Definitive diagnosis for all tumors of the urinary tract requires pathologic examination of biopsy material. In addition, routine medical practices and procedures might include: physical examination, radiographic examination, ultrasound scan, computer assisted tomography (CT) scan, lymphangiography, and other procedures for overall clinical evaluation of the patient.

Additionally, there are several products for the management of TCC/UT after first diagnosis for which there are clearances or approvals by the FDA.

V. Marketing History

The Matritech NMP22 Test Kit was approved by FDA on July 2, 1996 for use as an aid in the management of patients with transitional cell carcinoma of the urinary tract (TCC/UT)

after surgical treatment to identify those patients with occult or rapidly recurring TCC/UT. It is sold in the United States for this indication.

The Matritech NMP22 Test Kit, under export exemption, Section 801(e) of the Federal Food, Drug, and Cosmetic Act, has distributors for sales in the following countries: Algeria, Argentina, Australia, Austria, Belgium, Canada, Denmark, Egypt, Finland, France, Germany, Greece, Iceland, Israel, Italy, Japan, Korea, Morocco, Netherlands, New Zealand, Norway, People's Republic of China, Republic of South Africa, Russia, Spain, Sweden, Switzerland, Taiwan ROC, Tunisia, and Turkey

VI. Potential Adverse Effects of the Device on Health

When the Matritech NMP22 Test Kit is used as indicated, the adverse effects on the health of the patients being evaluated for transitional cell carcinoma of the urinary tract are associated with a false positive or negative test result. A false positive result may lead to more aggressive follow up procedures and possibly the initiation of therapy. A false negative result might lead to a delay in patient treatment.

VII. Summary of Studies

A. Nonclinical Laboratory Studies

The following performance characteristics were approved in the original PMA submission in July of 1996 for monitoring of bladder cancer and remain unchanged in the second supplement for diagnosis of bladder cancer.

- Limits of Detection = 2.1 U/mL
- Recovery = 89% - 111% with an overall mean of 99%
- Linearity of dilution was acceptable
- Study of potentially interfering substances

NCCLS Precision Study

This study assessed performance of the Matritech NMP22 Test Kit using the procedures outlined in the NCCLS guideline for precision studies EP5-A¹⁵.

TABLE 1: NCCLS Precision Study

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

These results are within acceptable limits for a device of this kind, although the percent coefficient of variation (% CV) of Specimen A is high.

Site To Site Reproducibility Study I

The purpose of this study was to demonstrate that comparable results were obtained when three different laboratories assayed matched urine samples using the Matritech NMP22 Test Kit. Three laboratories measured test precision using 3 controls and 3 panels of urine samples. Each laboratory assayed the 3 controls and the 3 specimen panels in replicates of four over four separate assays.

The results of the familiarization study were as follows. The within run %CV range was 0.2 to 8.2% for all but the specimen pool with the lowest NMP22 value. The corresponding overall %CV range for 20 assays was 5.6 to 9.34%. These results are within acceptable limits for a device of this kind. The overall %CV for the specimen pool with an average NMP22 value of 3.26, however, was 25.84%. This irreproducibility existed not only between laboratories but within each laboratory as well. The between-day %CV's for each of the 3 laboratories ranged from 13.7 to 27.9% for this low level specimen. This level is very high, especially, for a test with a proposed cut-off of 5 U/mL.

Site To Site Reproducibility Study II

After each laboratory completed the familiarization panel, 263 stabilized urine samples were assayed. The NMP22 values from Laboratory 2 and 3 were compared to Laboratory 1 (the central testing site) values and to each other. FDA subjected this paired data to three different analyses, all of which showed lack of site-to-site reproducibility.

TABLE 2: Comparison of group medians using the Wilcoxon Signed-Rank Test.

	Sample Size	Mean Difference	Median Difference	Range of Differences	p-value
Lab1 vs Lab2	263	0.2	0.2	(-15.1, 6.3)	0.0137
Lab1 vs Lab3	263	-0.5	-0.4	(-15.3, 6.1)	0.0002
Lab2 vs Lab3	263	-0.8	-0.6	(-11.6, 9.1)	0.0001

The p values showed a significant difference for all three comparisons using the Wilcoxon Signed-Rank Test of means and medians.

TABLE 3: Regression Method for comparison of site to site reproducibility

	Con-stant	Regres. Coeff.	p-Value to Test Const=0	95% Conf. Int. for Constant = 0	p-Value to Test Reg Coeff=1	95% Conf. Int. for Reg. Coeff.
Lab1 on Lab2	0.719	0.911	<0.0001	(0.40, 1.04)	<0.0001	(0.88,0.95)
Lab2 on Lab1	-0.204	0.992	0.245	(-.55, 0.14)	0.68	(0.95, 1.03)
Lab1 on Lab3	0.386	0.833	0.06	(0.00, 0.80)	<.0001	(0.79, 0.88)
Lab3 on Lab1	0.526	1.023	0.024	(0.07, 0.98)	0.38	(0.97, 1.08)
Lab2 on Lab3	-0.122	0.875	0.56	(-.53, 0.29)	<.0001	(0.83, 0.92)
Lab3 on Lab2	0.971	0.987	<.0001	(0.55, 1.39)	0.60	(0.94, 1.04)

For results to be reproducible, the slope should be 1.0 and the intercept 0. Regression analysis of slopes and intercepts meeting the stipulated criteria are in bold type. As can be seen, only the regression of Lab 2 on Lab1 satisfied both criteria.

TABLE 4: Analysis of Concordant and Discordant Pairs

NMP22 Cutoff		Sample Size	Concordance % (#)	Discordance % (#)
5 U/mL	Lab 1 vs. Lab 2	263	88.2% (232) upper boundary = 92%	11.8% (31)
	Lab 1 vs. Lab 3	263	85.5% (225) upper boundary = 89%	14.5% (38)
	Lab 2 vs. Lab 3	263	88.2% (232) upper boundary = 92%	11.8% (31)
10 U/mL	Lab 1 vs. Lab 2	263	95.8% (252) upper boundary = 98%	4.2% (11)
	Lab 1 vs. Lab 3	263	95.8% (252) upper boundary = 98%	4.2% (11)
	Lab 2 vs. Lab 3	263	93.9% (247) upper boundary = 96%	6.1% (16)

Results in table 4 show, the upper boundary of the one-sided 95% confidence limits did not reach 100% for either possible cutoff, 5 or 10 U/mL. All analyses of this data demonstrated questionable laboratory to laboratory reproducibility which is commonly seen in devices of this type. However, there were fewer discordant pairs at the 10 u/mL cutoff than 5.0 u/mL.

Non-refrigerated Centrifugation Study

A study to determine if non-refrigerated centrifugation causes temperature changes within stabilized NMP22 urine samples that compromise sample integrity showed that non-refrigerated centrifugation of stabilized samples was acceptable under the conditions of approximately 500 – 1000 x G for 10 to 15 minutes at room temperature (18-25° C).

Expiration Dating Extension Study

This study was conducted to extend dating for the Matritech NMP22 Test Kit components from the currently approved 18 months to 24 months. The protocol called for running 3 controls and specimen panels at four different levels of NMP22 monthly up to 24 months to look for significant differences or trends in the results. Analysis of the data failed to justify extension of the kit expiration date to 24 months.

Sample Shipping Extension Study

A study was conducted to determine whether extending the duration of shipping stabilized urine samples from three days (current package insert specification) to four days causes a statistically significant difference on the reported NMP22 value, and whether exposing stabilized urine to a temperature over 45°C causes a statistically significant difference on the reported NMP22 value.

Statistical analyses of the data demonstrated exposing stabilized urine to a constant elevated temperature of 35°C for 72 or 96 hours caused no statistically significant difference in the reported NMP22 values compared to those of the control condition of 72 hours at room temperature. The data justified the extension of the sample storage time from 72 to 96 hours at room temperature and 35°C in spite of the statistically significant difference appearing for the room temperature results.

Exposing the stabilized urine to a constant elevated temperature of 45°C resulted in statistically significant differences in NMP22 values. The draft Package Insert has been modified to recommend shipping samples frozen, if it is anticipated that they will be exposed to temperatures higher than 35°C during transport.

B. Clinical Investigations

A prospective clinical trial was conducted at 33 clinical sites with the following six objectives:

1. To determine the utility of using NMP22 levels in the differential diagnosis of patients with unresolved hematuria or other risk factors for urinary tract cancer.
2. To define the range of NMP22 levels in the urine of patients with benign diseases of the urinary tract.
3. To define the range of NMP22 levels in the urine of patients with newly diagnosed urinary tract cancer, stratified by stage and grade.
4. To define the sensitivity and specificity of this assay to detect newly diagnosed urinary tract cancer, stratified by stage and grade.
5. To define the range of NMP22 levels in the urine of normal, healthy persons.
6. To define the range of NMP22 levels in the urine of persons diagnosed with cancers other than the bladder and not yet receiving treatment.

Study Site Locations

Geographically diverse clinical sites performed the study. Investigators were encouraged to enroll all subjects who met entrance criteria and were willing to participate in an effort to include women and minority groups in representative numbers in the trial.

Subject Selection and Exclusion Criteria

Normal Healthy Volunteers. Three-hundred and twenty-nine (329) subjects over the age of 50 years with no significant current medical conditions and no known genitourinary diseases or conditions within the prior twelve months were recruited to be normal healthy volunteers. Individuals whose urine sample was trace positive or greater for blood as determined by dipstick at time of collection were not eligible for participation.

Patients at Risk of Bladder Cancer. Seven-hundred and sixty-nine (769) persons with unresolved hematuria and/or other symptoms or risk factors for urinary tract cancer, such as dysuria, exposure to carcinogens, or history of smoking were invited to participate in the RISK group. To have been included, patients must have undergone a diagnostic evaluation comprising voided cytology, cystoscopy (with biopsy if appropriate), and an upper tract imaging, such as intra vesicular pyelogram (IVP) or ultrasound, as part of their standard care.

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 malignancy 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 biopsied 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 tissue.

Patients were excluded from the analysis if they had one or more of the following protocol violations: no cystoscopy, cytology or upper tract imaging procedure (risk category); undocumented malignancy or active chemo-/radiation therapy (other cancer category); dipstick positive for hematuria (normal healthy volunteers); history of a urinary tract disease within the prior twelve months (normal healthy volunteers and other cancer category); or invalid urine sample, no informed consent, history of a prior cancer, or insufficient data (all categories).

All participants must have signed an IRB-approved informed consent. Patients or volunteers with a history of cancer of any type except non-melanomatous skin cancer were not eligible for inclusion in the study.

Patients with Other Cancers. Forty-nine (49) patients with clinically or pathologically confirmed malignancies other than of the urinary tract, for which they were not undergoing chemo-, immuno- or radiotherapy at the time of collection, and who had no known genitourinary diseases or conditions within the prior twelve months, were asked to participate in the OTHER CANCER group.

A total of 1147 Individuals from three categories were enrolled in the clinical trial from April 1998 until May 1999.

Study Population

Included in the analysis were 329 normal, healthy volunteers, 49 patients with cancers other than of the urinary tract, and 769 patients with unresolved hematuria or other symptoms or risk factors for urinary tract cancer, of which 56 were diagnosed with neoplasms, 448 with benign conditions and 265 with no urinary tract disease. Distribution of all risk patients was approximately equivalent between males and females, at 53% and 47% respectively, but of those patients diagnosed with a neoplasm (N=56), 85.7% were male. This is consistent with data from the American Cancer Society (Facts and Figures 1999) which indicates that bladder cancer is four times more common in men than in women. Mean age of cancer patients was 64.9 years, which is expected. Smoking is a major contributor of risk to the development of urinary tract cancer, so it is not surprising that 78.6% of the patients diagnosed with neoplasms had a history of smoking, versus 55.6% of risk patients who were diagnosed with no or benign urinary tract disease. African Americans comprise approximately 12% of the population across the nation, and therefore were well represented in this trial at 15% of the risk group.

The most common reason for inclusion of risk subjects into the clinical trial was unresolved hematuria (97.9%), with history of smoking the second (51.6%).

Subjects were enrolled in this study in a manner to ensure appropriate distributions by demographic variables of age, sex, race and smoking status. Adequate numbers were enrolled in each group to detect significant differences in NMP22 levels among groups and none were found.

Results and Analysis of the Study

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 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.

TABLE 5:

Percent Distribution of NMP22 (U/mL)							
	N	0-<7.5	7.5-10	>10-20	>20-50	>50-100	>100
Healthy Subjects							
Males > 50	111	88.3%	5.4%	4.5%	0.9%	0.9%	0%
Females > 50	218	85.8%	5.5%	6.0%	2.8%	0%	0%
Total	329	86.6%	5.5%	5.5%	2.1%	0.3%	0%
Benign Disease							
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%
Total Benign Disease*	448	77.2%	7.8%	7.4%	3.3%	1.3%	2.9%
Other Cancers							
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%
Total Other Cancers	49	89.8%	6.1%	0%	4.1%	0%	0%

*Some patients are included in more than one category

TABLE 6 shows the percent distribution of the NMP22 results for final diagnoses of the 769 patients who had symptoms or risk factors for TCC.

		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%	2.6%	0.4%	0.4%
	Benign Urinary Tract Disease	448	77.2%	7.8%	7.4%	3.3%	1.3%	2.9%
	TCC	56	37.5%	10.7%	12.5%	16.1%	7.1%	16.1%

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.

TABLE 7 shows sensitivity and specificity of NMP22 for this study for TCC using a cut-off of greater than or equal to 7.5 U/mL.

	Sensitivity (95% Exact Confidence Interval)	Specificity (95% Exact Confidence Interval)	PPV (95% Exact Confidence Interval)	NPV (95% Exact Confidence Interval)
NMP22* (cut-off >=7.5 U/mL)	62.5% (35/56) (48.5-75.1%)	78.0% (556/713) (74.8-81.0%)	18.2% (35/192) (13.0-24.4%)	96.4% (556/577) (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.

TABLE 8 shows the sensitivity and specificity of voided urine cytology for this study for TCC.

TABLE 8: Voided Cytology: Sensitivity, Specificity, PPV and NPV for Risk factor Patients (T0-T4)				
	Sensitivity (95% Exact Confidence Interval)	Specificity (95% Exact Confidence Interval)	PPV (95% Exact Confidence Interval)	NPV (95% Exact Confidence Interval)
Voided Cytology*	32.6% (15/46) (19.5-48.0)	100% (713/713) (99.5-100)	100% (15/15) (78.2-100)	95.8% (713/744) (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.

TABLES 9, 10 and 11 compare the NMP22 results (cut-off = 7.5 U/mL) to cytology results and the combination of NMP22 and cytology for the different stages and grades of TCC.

TABLE 9: Percent and Fraction of Positives by stage(T0 ¹⁵ -T4) (95% confidence interval)			
	NMP22 Cut-off \geq 7.5 U/mL	Voided Cytology	NMP22 & Cytology Combined*
T0 ¹⁵	60.0% (3/5) (14.7-94.7%)	0% (0/5) (-)	60.0% (3/5) (14.7-94.7%)
Ta	45.0% (9/20) (23.1-68.4%)	16.7% (3/18) (3.6-41.4%)	57.9% (11/19) (33.5-79.8%)
Tis	80.0% (4/5) (28.4-99.5%)	66.7% (2/3) (9.4-99.2%)	100% (4/4) (39.8-100%)
T1	63.6% (7/11) (30.8-89.1%)	50.0% (5/10) (18.7-81.3%)	72.7% (8/11) (39.0-94.0%)
T2, T3, T4	76.9% (10/13) (46.2-95.0%)	55.6% (5/9) (21.1-86.3%)	92.3% (12/13) (64.0-99.8%)
Tx	100% (2/2) (15.8-100%)	0% (0/1) (-)	100% (2/2) (15.8-100%)

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

	NMP22 Cut-off ≥ 7.5 U/mL	Voided Cytology	NMP22 & Cytology Combined*
No malignancy	60.0% (3/5) (14.7-94.7%)	0% (0/5) (-)	60.0% (3/5) (14.7-94.7%)
Low	50.0% (9/18) (26.0-74.0%)	13.3% (2/15) (1.7-40.5%)	56.3% (9/16) (29.9-80.3%)
Medium	70.6% (12/17) (44.0-89.7%)	42.9% (6/14) (17.7-71.1%)	82.4% (14/17) (56.6-96.2%)
High	68.8% (11/16) (41.3-89.0%)	58.3% (7/12) (27.7-84.8%)	87.5% (14/16) (61.7-98.5%)

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

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%

As indicated from the above analysis, urinary NMP22 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 values below 7.5 U/mL are less likely to have TCC.

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

VIII. Conclusions Drawn from the Studies

The performance specifications for the Matritech NMP22 Test Kit submitted in the original PMA submission met the usual specifications for an immunoassay performed in a licensed clinical laboratory. This included precision, reproducibility, recovery, stability for claimed time periods, linearity, and limits of detection. It was noted, however, that the limits of detection derived from two standard deviations above the mean of 20 replicates of the zero calibrator, was not very distant from the sponsor's proposed diagnostic test cutoff of 5 U/mL.

The results of the NCCLS precision study showed all of these results within acceptable limits for a device of this kind. It was noted, however, that the total %CV for one sample at a mean NMP22 value of 6.3 U/mL was >10%. This finding called into question the reproducibility of the product at the sponsor's recommended diagnostic test cutoff of 5 U/mL.

The results of a site-to-site reproducibility study showed all results within acceptable limits for a device of this kind with the exception of the lowest sample pool with a mean of 3.6 U/mL. The high %CV for this sample pool was a second indicator of possible problems with the test design at low NMP22 values. This is a common occurrence for samples near the limit of detection of a test (2.1 U/mL). However, the mean of the majority of all populations with normal levels falls near 3.26 U/mL, and the diagnostic test cutoff proposed by Matritech was nearby at 5 U/mL.

The results of the 263 clinical sample site-to-site reproducibility study analyzed by FDA also indicated lack of site-to-site reproducibility. It confirmed a trend of increased imprecision at low levels of NMP22. After considerable deliberation, the panel concluded that the test was not optimized for a cutoff at 5 U/mL, because it was very close to the limit of detection at 2.1 U/mL, the irreproducibility was unacceptably high at low NMP22 values, and the lowest positive calibrator was at 7.5U/mL, above the proposed cutoff of 5 U/mL. This information and data prompted the panel to recommend as one of the two condition for approval the moving of the test cutoff upwards to 7.5 U/mL where the lowest positive calibrator is located.

Preclinical study results confirmed that non-refrigerated centrifugation of stabilized samples was acceptable under the conditions of approximately 500 – 1000 x G for 10 to 15 minutes at room temperature (18-25° C).

The FDA decided that insufficient data analysis had been performed to extend expiration to 24 months. It was therefore recommended that the kit expiration date remain at 18 months until further data analysis provides justification for extension of the expiration date.

The Sample Shipping Extension Study provided data to justify the extension of the sample storage time from 72 to 96 hours at room temperature and 35°C.

Exposing the stabilized urine to a constantly elevated temperature of 45°C resulted in statistically significant differences in NMP22 values. The draft Package Insert has been modified to recommend shipping samples frozen, if it is anticipated that they will be exposed to temperatures higher than 35°C during transport.

The clinical studies defined the ranges of NMP22 values in normal, healthy persons, in persons with cancers other than those of the urinary tract, in persons at risk of bladder cancer with benign urinary tract conditions and in persons newly diagnosed with bladder cancer. The cutoff recommended by the Immunology Devices Panel on December 13, 1999 consistent with the test performance characteristics was 7.5 U/mL. The per cent

positivity for the various stages and grades of bladder cancer found in the clinical study were also determined and compared to those of voided urine cytology and for use as an adjunct to cytology.

The findings of the clinical studies indicated that NMP22 has potential use as an aid to diagnosis of bladder cancer. An NMP22 value less than 7.5 U/mL indicated that a patient had lower risk for presence of bladder cancer. An elevated NMP22 value (≥ 7.5 U/mL), on the other hand, did not necessarily indicate that a patient had active malignant disease (78 percent specificity in the patient group). Further follow-up of positive results is necessary to determine if bladder cancer is present. Other conditions, beside bladder cancer, may cause elevations in NMP22 values, particularly some benign urinary tract conditions, such as urinary tract infections or cystitis, following cystectomy in patients with either a neobladder or ileal conduit, and in patients receiving systemic chemotherapy. For these reasons, the Immunology Devices Panel recommended that the test be used in conjunction with and not in lieu of current diagnostic procedures.

CDRH concurred with the Panel recommendations and has concluded that the device is safe and effective when used ~~as intended~~ for the quantitative measurement of NMP22 in stabilized voided urine in patients with symptoms or risk factors for transitional cell cancer of the bladder in conjunction with and not in lieu of current standard diagnostic procedures.

Risk/Benefit Analysis

Since the Matritech NMP22 Test 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 NMP22 assay is low, but the benefit of identifying patients early malignancy is increased.

IX. Panel recommendation

The Immunology Devices Panel recommended at the panel meeting on December 13, 1999 that the PMA for the Matritech NMP22 Test Kit was approvable with conditions and recommended the following two conditions:

1. Amend the intended use of the test for diagnosis to add: "...to be used in conjunction with and not in lieu of current standard diagnostic procedures."
2. To employ a cutoff of 7.5 U/mL until sufficient calibrators and controls are incorporated into the assay to justify lowering the cutoff to the proposed 5 U/mL.

X. CDRH Action on the Application

CDRH concurred with the recommendations of the Panel. Matritech, Inc. responded to the conditions by submitting the requested labeling changes in the form of an Amendment received by FDA on January 10, 2000. CDRH issued an approval order for the applicant's PMA for the Matritech NMP22 Test Kit on

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The applicant's manufacturing and control facilities were inspected on May 1, 1997 and the facilities were found to be in compliance with the Good Manufacturing Practice Regulations (GMP). The shelf-life of the Matritech NMP22 Test Kit has been established at 18 months.

XI. Approval Specifications

Directions for use: See attached labeling

Conditions of Approval: CDRH approval of this PMA is subject to full compliance with the conditions described in the approval order (Attachment B).

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