## Comparison and Sensitivity Studies

## Performance Evaluation Studies

## Sources of Urine Specimen

The sources of the urine used (confirmed bladder cancer subjects) in sensitivity testing of the Bladder Tumor Test Panel vs. Cytology (gold-standard) biopsy are listed below

1. Central Laboratory of Hospital A

This hospital has 650 beds with several medical departments. The central laboratory of the hospital has 25 medical laboratory assistants and analyses on a routine basis approximately 2000 patient samples in the area of coagulation, drug monitoring, hematology, endocrinology, infectious disease, pre-natal analyses, microbiology, hormone analyses and histology.

2. Central Laboratory of Hospital B

The hospital has 870 beds with several medical departments. The central laboratory of the hospital has 33 medical laboratory assistants and analyses on a routine basis approximately 3000 patient samples in the area of coagulation, drug monitoring, hematology, endocrinology, infectious disease, pre-natal analyses, microbiology, hormone analyses and histology.

3. Central Laboratory of Hospital C

The hospital has 500 beds with several medical departments. The central laboratory of the hospital has 17 medical laboratory assistants and analyses on a routine basis approximately 1000 patient samples in the area of coagulation, drug monitoring, hematology, endocrinology, infectious disease, pre-natal analyses, microbiology, hormone analyses and histology.

4. Central Laboratory of Hospital D

The hospital has 500 beds with several medical departments. The central laboratory of the hospital has 21 medical laboratory assistants and analyses on a routine basis approximately 1000 patient samples in the area of coagulation, drug monitoring, hematology, endocrinology, infectious disease, pre-natal analyses, microbiology, hormone analyses and histology.

## Relative Sensitivity

The diagnostic performance of the test was evaluated in a randomized controlled clinical trial. Here, a comparison study was performed with cytology (gold-standard, biopsy confirmed).

A total of 225 patients with biopsy confirmed bladder cancer took part in this study, of the 225 patients, stages were determined on 223, and grades are determined on 210 patients.

The age range of these 225 individuals was from 63 to 81 years, 86% were male. The collected specimens were tested in the one step bladder tumor test and compared with Cytology performed on the patient. A positive line from either Hemoglobin or BCA test zone of one step Bladder Tumor test is considered positive specimen.

All comparison studies were conducted blindly, i.e. the laboratory technician did not know which samples was HB/BCA positive or negative before testing.

**Table 0-1: Relative Sensitivity of the Bladder Tumor test in comparison with Cytology test (n=225)**

| Stage | N | Sensitivity (BCA Only) | Sensitivity (BCA and HB) |
|---|---|---|---|
| Ta | 14 | 57% | 57% |
| T1 | 69 | 82.6% | 85.5% |
| T2, T3 | 140 | 86.4% | 94.2% |
| Grade | N | | |
| 1 | 47 | 38.2% | 42.5% |
| 2 | 61 | 63.9% | 70.5% |
| 3 | 102 | 89.2% | 97.1% |

**Conclusion:** The sensitivity of the Bladder Tumor test is a function of tumor stages and grades, Varies from 57% to 97% sensitivity (the sensitivities obtain from this study is similar to other published studies where the same marker, hCFHrp were used.

**Specificity**

The specificity is defined as the probability to have a negative result in the absence of the particular condition. The specificity was assessed by studying specimen samples from urine (hemoglobin/BCA) negative subjects containing no other closely related substance. Specimen samples that were negative to HB/BCA were spiked with other substance. These samples were tested using the Bladder Tumor test by a replicate of 10. A sample was classified negative, when no purple color band was visible for the Test Line but the purple color “C” control line being visible within 10 minutes. A sample was classified positive, when both the control and test line were visible within 10 minutes (results see Table 0-1).

**Table 0-1:** Specificity testing of the Bladder Tumor test using negative and positive control samples spiked with various substances.

| Number of Tests | Samples spiked with following substances | Negative Sample | Positive Sample (hemoglobin and BCA ) |
|---|---|---|---|
| | | **Bladder Tumor Test**<br><br>(visible test and control band on test cassette strip = positive) | **Bladder Tumor Test**<br><br>(visible test and control band on test cassette strip = positive) |
| **10** | Acinetobacter calcoaceticus | All (-) | All (+) |
| **10** | Proteus vulgaris | All (-) | All (+) |
| **10** | Salmonella typhi | All (-) | All (+) |
| **10** | Acinetobacter spp | All (-) | All (+) |
| **10** | Staphylococcus aureus | All (-) | All (+) |
| **10** | Candida albicans | All (-) | All (+) |
| **10** | Neisseria gonorrhae | All (-) | All (+) |
| **10** | Escherichia coli | All (-) | All (+) |
| **10** | Neisseria catarrhalis | All (-) | All (+) |
| **10** | Gardnerella vaginalis | All (-) | All (+) |
| **10** | Neisseria meningitidis | All (-) | All (+) |
| **10** | Streptococcus faecalis | All (-) | All (+) |
| **10** | Neisseria lactamica | All (-) | All (+) |
| **10** | Streptococcus faecium | All (-) | All (+) |
| **10** | Pseudomonas aeruginosa | All (-) | All (+) |
| **10** | Trichomonas vaginalis | All (-) | All (+) |

## Interference Studies

Potentially interfering chemical substances such as pain medication, lipids, bilirubin and glucose were supplemented to clinically defined negative normal specimens and clinically defined positive specimens. These samples were tested using the Bladder Tumor test by a replicate of 10. A sample was classified negative, when no purple color band was visible for the test line but the purple color "C" control line being visible within 10 minutes. A sample was classified positive, when both the control and test line were visible within 10 minutes.

**Table 0-1: Interference studies with the Bladder Tumor test using clinically defined Bladder Cancer negative samples supplemented with various potential interfering substances.**

| # Tests done | Clinically defined negative Tumor Cancer specimen<br><br>supplemented with following substances | Bladder Tumor Test<br><br>Test result |
|---|---|---|
| 10 | Acetamiophen, 20 mg/dl | All (-) |
| 10 | Acetyl salicylic Acid, 20 mg/dl | All (-) |
| 10 | Ascorbic Acid, 20 mg/dl | All (-) |
| 10 | Atropine, 20 mg/dl | All (-) |
| 10 | Bilirubin, 60 mg/dl | All (-) |
| 10 | Caffeine, 20 mg/dl | All (-) |
| 10 | Creatinine, 20 mg/dl | All (-) |
| 10 | Gentesic Acid, 20 mg/dl | All (-) |
| 10 | Glucose, 2000 mg/dl | All (-) |
| 10 | Ketones, 40 mg/dl | All (-) |
| 10 | Mestranol, 3 mg/dl | All (-) |
| 10 | Nitrite, 20 mg/dl | All (-) |
| 10 | Penicillin, 40,000 U/dl | All (-) |
| 10 | Sodium Heparin, 3 mg/dl | All (-) |
| 10 | Lithium Heparin, 3 mg/dl | All (-) |

In conclusion, none of the above tested substances showed any interferences with neither a clinically defined negative nor a positive specimen. Negative specimen samples with supplementation of potentially interfering substances gave consistently negative test results, whereas specimen samples positive to Bladder Tumor scored consistently positive.

## Conclusion:

**Conclusion:** The sensitivity of the Bladder Tumor test is a function of tumor stages and grades, Varies from 57% to 97% sensitivity. The addition of HB parameter improves the sensitivity of the test specially in later stage and high grade cases, where blood is typically present in urines.

## Read Time Studies

Read Time Study; Remove the test disk from the foil packet, and place it on a flat, dry surface. Holding the sample dropper above the test disk, squeeze 2 to 3 drops of specimen into the sample wells. As the test begins to work, we see purple color move across the result window in the center of the test disk and calculate the time. The read time for control and test line is summarized as in Table 0-1.

For the purpose of read time study only, the weak positive, positive and strong positives are listed:

Weak Positive for BCA is defined as 250ng/ml and HB as 500ng/ml

Positives for BCA is defined as 1000ng/ml and HB as 2000ng/ml

Strong Positive for BCA is defined as 10,000ng/ml and HB as 20,000ng/ml

**Table 0-1: Read Time Study**

| | Specimen | Control Line<br>(in minutes, average) | Test Lines (T)<br>(in minutes, average) |
|---|---|---|---|
| 5 | negative | + (3) | N |
| 5 | weak positive | + (3) | P (10) |
| 5 | positive | + (3) | P (5) |
| 5 | strong positive | + (3) | P (2) |

Conclusion; Read time for Control Line are 3 minutes and for Test Line are 5 to 10 minutes.

## Reproducibility Study

The reproducibility study was carried in three different sites. Negative, weak positive and positive specimen samples had been tested by total 120 Bladder Tumor test kit. The samples were tested two times in the same day, and in two different assays, each day for 20 days. The study results are shown in Table 0-1.

**Table 0-1: Reproducibility Study**

| | **Bladder Tumor Test** | | |
|---|---|---|---|
| **Days** | Negative | Weak Positive | Strong Positive |
| | **Test Result (number of tests)** | | |
| **1** | NEG (2) | POS (2) | POS (2) |
| **2** | NEG (2) | POS (2) | POS (2) |
| **3** | NEG (2) | POS (2) | POS (2) |
| **4** | NEG (2) | POS (2) | POS (2) |
| **5** | NEG (2) | POS (2) | POS (2) |
| **6** | NEG (2) | POS (2) | POS (2) |
| **7** | NEG (2) | POS (2) | POS (2) |
| **8** | NEG (2) | POS (2) | POS (2) |
| **9** | NEG (2) | POS (2) | POS (2) |
| **10** | NEG (2) | POS (2) | POS (2) |
| **11** | NEG (2) | POS (2) | POS (2) |
| **12** | NEG (2) | POS (2) | POS (2) |
| **13** | NEG (2) | POS (2) | POS (2) |
| **14** | NEG (2) | POS (2) | POS (2) |
| **15** | NEG (2) | POS (2) | POS (2) |
| **16** | NEG (2) | POS (2) | POS (2) |
| **17** | NEG (2) | POS (2) | POS (2) |

| | | | |
|---|---|---|---|
| **18** | NEG (2) | POS (2) | POS (2) |
| **19** | NEG (2) | POS (2) | POS (2) |
| **20** | NEG (2) | POS (2) | POS (2) |

Conclusion: The kits were tested two times in the same day, and in two different assays each day for 20 days. This permits separate tests of between day, between-assay and within-day which they are shown consistent results.

The reproducibility study was performed at three different sites. The test results are shown Table 0-2.

**Table 0-2: Reproducibility Study Based on Study Sites**

| | **Bladder Tumor Test** | | |
|---|---|---|---|
| **Test sites** | Negative | Weak Positive | Strong Positive |
| | **Test Readings ( number of tests)** | | |
| **1** | Neg (40) | Pos (40) | Pos (40) |
| **2** | N eg(40) | Pos (40) | Pos (40) |
| **3** | Neg (40) | Pos (40) | Pos (40) |

Conclusion: The tests results of three sites were in 100% agreement.