P-5916

Status BTA

For Professional *In Vitro* Use

A Rapid Test For the Qualitative Detection of Bladder Tumor Associated Antigen in Human Urine

Caution: U.S.A. 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.

LifeSign, LLC

Catalog No.	39010	10 Test Kit
Catalog No.	39025	25 Test Kit

Intended Use

The ***Status BTA*** test is an *in vitro* immunoassay intended for the qualitative detection of bladder tumor associated antigen in urine of persons diagnosed with bladder cancer. This test is indicated for use as an aid in the management of bladder cancer patients in conjunction with cystoscopy.

Summary and Explanation

Bladder Cancer is the fourth most common form of cancer in men and ninth most common form in women in the United States[1]. Approximately 75 to 85% of these patients present with transitional cell carcinoma (TCC) confined to the superficial mucosa of the bladder[2]. The risk of recurrence in these patients is 75%. Patients with previous diagnosis of bladder cancer have been routinely followed for recurrence by urine cytology and cystoscopy. Both methods have their limitations.

Cystoscopy is considered the diagnostic standard for sensitivity and specificity when a biopsy is not obtained. This method is an invasive procedure associated with patient discomfort, is expensive and is limited to diagnosis of those tumors that can be visualized[3].

Voided urine cytology (VUC), or the examination of urinary sediment for cancer cells, has several characteristics that contribute to suboptimal results. Urothelial cells require about a year to replicate, so few are available for examination in any particular sample[3]. Exfoliated cells enter a hostile environment of high acidity and low osmolality which may obscure essential diagnostic features. Standards for specimen collection, preservation, processing and interpretation have not been widely accepted. Routine cytology, as a monitoring tool exhibits variable sensitivity depending on the tumor stage and grade with lowest sensitivity reported for early stage disease[4,5]. In addition, the best quality results are obtained from examination of samples collected under specific voiding procedures[4].

The management of patients with bladder cancer could be improved with a rapid, simple, urine test that could be performed at point of care or in the laboratory. Recent studies have shown that the ***Status BTA*** test, which qualitatively detects bladder tumor associated antigen can be extremely useful in this regard[6,7]. The ***Status BTA*** test is a single-step, antibody based test which is performed in only 5 minutes with no pretreatment of the urine sample.

Bladder Tumor Associated Antigen

The monoclonal antibodies used in the ***Status BTA*** test were generated against urine components from patients with histologically confirmed bladder cancer. Bladder tumor associated antigen was identified as human complement factor H related protein (hCFHrp) similar in composition, structure and function to human complement factor H (hCFH)[8,9]. hCFH, which is also recognized by the monoclonal antibodies utilized in the ***Status BTA*** test, is found in human plasma at concentrations of approximately 480 µg/mL. In cell culture, hCFHrp was shown to be produced by several human bladder cancer cell lines, but not by normal epithelial cell lines[8,9]. Using *in situ* hybridization methods in tumor specimens, hCFHrp was shown to be produced by cancer cells and macrophages but not by normal epithelia.

hCFH plays a key inhibitory role in the control of the alternative complement pathway that functions to lyse cells recognized as foreign to the host. By interaction with complement factor C3b, hCFH serves to inhibit the formation of a membrane attack complex, thereby preventing cell lysis[10]. *In vitro*, bladder tumor associated antigen interrupts the complement cascade and protects cells from lysis by complement. This inhibitory effect can be reversed by the use of monoclonal antibodies specific for hCFHrp[11]. Production of bladder tumor associated antigen may confer a selective growth advantage to cancer cells *in vivo* by allowing the cells to evade the host immune system.

Principle of the Procedure

The ***Status BTA*** test is an immunoassay utilizing two different monoclonal antibodies (MAbs) to specifically detect the presence of bladder tumor associated antigen in urine. Each MAb specifically binds to a different epitope on the target antigen (hCFHrp). One MAb serves as the hCFHrp capture agent. The second MAb is conjugated to colloidal gold and serves as the reporter molecule if hCFHrp is present in the specimen.

Patient urine is added to the sample well of the device and allowed to react with the colloidal gold conjugated reporter antibody. If the antigen is present in the sample, it will interact with the conjugate to form an immune complex. The reaction mixture flows through the membrane which contains zones of immobilized antibodies. In the Test Position (T), antigen-conjugate complexes are trapped by the capture antibody, forming a visible line. In the absence of the antigen in the patient urine, no visible line will form. The Control Position (C) zone contains an immobilized goat anti-mouse IgG-specific antibody which will capture the conjugated antibody independently of the presence or absence of the antigen, thereby always producing a line. This procedural control assures the operator that each device is working properly.

Contraindications

- Do not use beyond the labeled expiration date.
- Do not reuse disposable test devices. Discard after single use.
- Do not use if pouch is damaged or opened.
- Do not touch the membrane located within the window.

Warning and Precautions

- For *in vitro* diagnostic use only.
- To avoid cross-contamination of samples, use a new dropper (provided with each device) for each patient urine.
- Treat urine samples and used devices as if they are potentially infectious.

Devices and Reagents

Status BTA test device - individually packaged in a sealed foil pouch with a transfer pipette and a desiccant. Each device incorporates a membrane-immobilized murine anti-hCFHrp capture MAb and a conjugated murine anti-hCFHrp MAb in a protein matrix containing sodium azide. The procedural Control zone contains an immobilized goat anti-mouse IgG-specific antibody in a protein matrix containing sodium azide.

Storage and Stability

Store the ***Status BTA*** test kit at 2 - 30°C. Do not freeze. The test kit is stable when stored at these temperatures until the expiration date printed on the box label.

Indications of Device Deterioration

If a ***Status BTA*** test device fails to produce a line in the Control Position (C) when used according to the Patient Test Procedure, the test is invalid and must be repeated with a new device.

Specimen Collection, Storage and Preparation

Voided urine or urine from a catheterized patient is required for the ***Status BTA*** test. Bladder barbotage specimens, serum, plasma or whole blood should not be used. Urine should be collected without preservatives or fixatives in a clean urine cup and labeled appropriately. If urine is to be used for other tests, remove an aliquot of the specimen (a minimum of 2 ml) for this test to avoid contamination. Swirl to mix the urine before testing. Urine samples may be stored at room temperature for up to 48 hours after collection. If the urine sample is not tested within 48 hours, it should be refrigerated at 2 - 8°C for up to 7 days. If the refrigerated urine sample is not tested within 7 days, it should be stored at or below -20°C until tested. A frozen sample is stable for 24 weeks at -20°C including up to 4 freeze/thaw cycles.

- Do not use paper or foam cups for urine specimen collection or storage.
- The effect of radiation therapy or systemic chemotherapy on the ***Status BTA*** test is unknown.
- The effect of treatment with intravesical agents, such as BCG, mitomycin C, Thiotepa, bropiramine (investigational) or interferon (investigational), is unknown.
- The antigen concentration variation in first morning urine specimens has not been determined.
- The effects of experimental drugs on the ***Status BTA*** test are unknown.
- Some patients with benign renal disease (such as stones and nephritis), urinary tract infections, cystitis, sexually transmitted diseases or renal cancer including upper tract TCC may yield positive results with the ***Status BTA*** test.
- For trauma to the bladder or urinary tract due to surgery, biopsy, etc., the physician should allow ample time for trauma recovery before using the test.

Contents of Kit

Status BTA **10-Test Kit**
(Item #39010) Components:

- 10 Foil Packages. Each package contains -
  1 ***Status BTA*** device
  1 Transfer pipette
  1 Disposable desiccant pouch
- 1 Package Insert

Status BTA **25-Test Kit**
(Item #39025) Components:

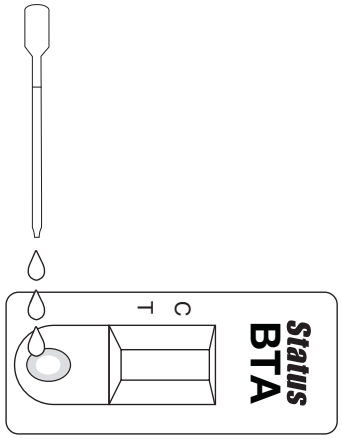
- 25 Foil Packages. Each package contains -
  1 ***Status BTA*** device
  1 Transfer pipette
  1 Disposable desiccant pouch
- 1 Package Insert

Materials Required But Not Provided

- Timer
- Urine collection container (do not use paper or styrofoam cups)
- Positive and Negative External Controls, e.g. ***Status BTA*** Test Control Kit (item #200019)

Patient Test Procedures

1) Bring test materials and patient urine sample to room temperature (17 - 37°C, 63 - 99°F). Gently swirl to mix patient's urine sample.



2) Remove the test device and transfer pipette from foil package. Throw away small desiccant pouch. Place the device on a clean, well-lit, flat surface and label with the patient's identification.

3) Fill the transfer pipette provided with the patient's urine sample and hold it upright above the sample well as shown.

4) Allow 3 FULL drops (without air bubbles) to fall into the sample well. Start timer.

5) When timer reaches 5 minutes, read results within 1 minute. Read results as shown under "Interpretation of Results."

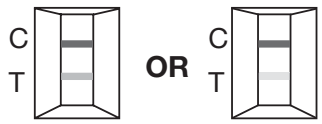
Read at 5 minutes but NO LATER THAN 6 MINUTES. Test result is not valid if read after 6 minutes.

6) Discard used transfer pipette and test device in a proper biohazard container.

Interpretation of Results

1) Check the Control Position (C). A pink or reddish-brown line must appear for the test to be valid.

2) **Positive Result:** Carefully look at Test Position (T). ANY pink or reddish-brown colored line, NO MATTER HOW FAINT, in the Test Position (T) is a positive result. Neither the intensity nor the color should be compared to that seen in the Control Position (C).

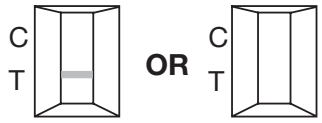


(examples of positive results)

3) **Negative Result:** Carefully look at Test Position (T). No colored line in the Test Position (T) is a negative result.

(example of negative results)

4) **Invalid Test Result:** If no line appears in the procedural Control Position (C), the test is invalid and must be repeated with a new device. The most common reason for an invalid test result is failure to add exactly 3 FULL drops.



(examples of invalid results)

Quality Control

Good laboratory practices recommend the use of appropriate controls. There are two types of controls for the ***Status BTA*** test - the internal procedural control and external controls.

Procedural Control

The procedural Control is found in the Control Position (C) of the test device. This control assures the operator that (A) sample addition and migration through the device has occurred and that (B) the control goat anti-mouse antibody and the reporter MAb antibody are intact and functional. This control does not ensure that the Test Position (T) is accurately detecting the presence or absence of bladder tumor associated antigen in the sample.

External Controls

External controls are used to assure the operator that the capture and conjugated antibodies are present and reactive. External controls will not detect an error in performing the patient test procedure. The ***Status BTA*** Test Control Kit is available separately and contains Positive and Negative Control solutions (item #200019).

If controls do not perform as expected, do not use the test results. Repeat the test or call Technical Service at 800-526-2125.

Limitations

Results of the ***Status BTA*** test should not be interpreted as absolute evidence for the presence or absence of bladder cancer. Any disease which could cause endogenous hCFH to leak into the bladder may cause a positive test result. Positive results have been observed in some patients with renal stones, nephritis, renal cancer (including upper tract TCC), urinary tract infections, cystitis, sexually transmitted diseases and recent trauma to the bladder or urinary tract.

The ***Status BTA*** test should not be used as a screening test for individuals without biopsy confirmed bladder cancer. The result from the ***Status BTA*** test should be used only in conjunction with information available from the clinical evaluation of the patient and other diagnostic procedures.

Expected Values

CLINICAL SENSITIVITY

Status BTA test sensitivity (Table I) was determined using urine samples from 220 patients with biopsy confirmed bladder tumor recurrence. Samples were collected from 5 different geographic locations throughout the United States and stored frozen until tested. Testing of samples for this study was performed at Alidex, Inc. The average patient age was 68 years, 79% were males, 67% Caucasian, 1% African American, 4% Asian, Hispanic or other, and 27% of unknown ethnicity. Results are presented below by stage and by grade of the tumor.

Table I. *Status BTA* TEST SENSITIVITY BY STAGE AND GRADE*

STAGE	N	SENSITIVITY(%)
Ta	111	51
T1	38	90
≥ T2	50	88
Tis	18	61
GRADE	**N**	**SENSITIVITY(%)**
1	57	42
2	56	66
3	95	83

*3 patients without stage and 12 without grade determinations.

Table II presents the overall sensitivity in 220 patients with histologically confirmed bladder cancer recurrence (Table I), as well as the specificity in 107 patients who were being monitored for recurrence of bladder cancer and determined by cystoscopy and/or biopsy to have no evidence of disease (NED) at the time of the *Status BTA* test determination.

Table II. *Status BTA* TEST RESULTS FROM PATIENTS WITH A HISTORY OF BLADDER CANCER

		Status BTA		
		POSITIVE	NEGATIVE	TOTAL
HISTOLOGY/ CYSTOSCOPY RESULT	POSITIVE	147	73	220
	NEGATIVE	32	75	107
	TOTAL	179	148	327

Monitoring sensitivity = 67% (60-73,95% confidence interval)
Monitoring specificity = 70% (61-79,95% confidence interval)

Using the data in Table II and a 10%, 20%, and 30% hypothetical prevalence of bladder cancer recurrence, the positive predictive values and negative predictive values of the ***Status BTA*** test are presented in Table III. Due to the possibility that bladder cancer may have been present in some of the NED individuals in this study, yet missed by cystoscopy, the true specificity in these patients and the positive and negative predictive values are likely to be higher.

Table III. HYPOTHETICAL POSITIVE PREDICTIVE VALUES (PPV) AND NEGATIVE PREDICTIVE VALUES (NPV) OF THE *Status BTA* TEST

BLADDER CANCER RECURRENCE PREVALENCE	PPV	NPV
10%	19.8	95.0
20%	35.8	89.4
30%	48.8	83.1

A subset of the patients with histologically confirmed bladder cancer (131) also had voided urine cytology (VUC) performed on the same sample as the Status BTA test (Table IV). The ***Status BTA*** test was shown to be more sensitive than VUC in all categories except for Tis (tumor in situ).

Table IV. *Status BTA* TEST AND VUC SENSITIVITIES

STAGE	N	SENSITIVITY STATUS BTA (%)	SENSITIVITY VUC (%)	SENSITIVITY STATUS BTA +VUC (%)
Ta	73	45	7	49
T1	27	85	41	85
≥ T2	16	75	38	81
Tis	15	53	60	80

In a subset of patients (99) with a history of bladder cancer and no evidence of disease the specificity of the Status BTA test was 69% compared to VUC with a specificity of 97%.

CLINICAL SPECIFICITY

Status BTA test specificity (Table V) was determined using urine samples from 555 individuals with no history of bladder cancer. Samples were collected from 5 different geographic locations throughout the United States and stored frozen (-80°C) until tested. Testing of samples for this study was performed at Alidex, Inc. The average age was 55 years, 52% were females, 86% were Caucasian, 8% African American, 4% Asian, Hispanic or other, and 3% of unknown ethnicity. The normal healthy population consisted of 60% non-smokers. The non-genitourinary (GU) diseases and cancers (71% of samples provided by females) included diabetes, arthritis, lupus erythematosus and other collagen degenerative diseases, as well as leukemia, lymphomas, breast, lung and gastrointestinal cancers. The non-bladder genitourinary cancers category (69% of samples provided by males) consisted of prostate, renal cell, renal TCC, endometrial, ovarian and other GU carcinomas. The GU disease category (52% of samples provided by females) consisted of patients with benign prostatic hyperplasia (BPH), prostatitis, urethritis, renal stones and disease, urinary tract infections (UTI), incontinence, sexually transmitted diseases (STD) and other disorders.

The results indicated that in healthy individuals and individuals without GU diseases and malignancies, the BTA stat test negative rate was 95% and 93%, respectively. Positive ***Status BTA*** test results may occur in patients with renal disease such as stones and nephritis and patients with renal cancer including upper tract TCC. Expected results may vary depending on the patient population tested.

Table V. *Status BTA* TEST SPECIFICITY RESULTS

	NUMBER OF SUBJECTS	TEST NEGATIVE (%)
Healthy Subjects	**167**	**95**
Non-Smokers	100	93
Smokers	67	97
Non-Genitourinary Benign Diseases and Cancer	**105**	**93**
Non- Genitourinary Benign Diseases	52	98
Non-Genitourinary Cancer	53	89
Genitourinary Diseases	**152**	**72**
BPH	26	88
Benign Renal Disease	32	50
Misc. GU Disease	94	76
UTI/cystitis	30	60
STD	24	79
Other	40	85
Genitourinary Trauma	**77**	**73**
Prostate Cancers	45	78
Renal Cancers	7	29
Renal TCC	1	0
Renal Cell Carcinoma	6	33
Other Cancers	25	76
Genitourinary Trauma	**54**	**33**
TOTAL[A]	**555**	**NA**
History of Bladder Cancer - No Evidence of Disease[B]	**107**	**70**

[A] total of subjects with no history of bladder cancer

[B] No evidence of disease confirmed by cystoscopy and/or biopsy; 78% of patients in this category were males

Performance Characteristics

HIGH DOSE HOOK EFFECT

High dose hook (prozone) effect tests were conducted to determine if the ***Status BTA*** test is free from interference from high concentration positive patient samples. Results showed that there was no prozone effect up to 12,400 U/mL bladder tumor associated antigen in a patient's urine sample, which was the highest concentration available for testing.

REPRODUCIBILITY

Three lots of ***Status BTA*** devices were used for the reproducibility studies to determine day-to-day, reader-to-reader and lot-to-lot variability. These studies were conducted by testing 10 replicates of 4 blinded samples per day for 5 days using three independent readers for each lot of devices. Between laboratory reproducibility studies were conducted at three laboratories by testing 10 replicates of 4 blinded samples on one lot of ***Status BTA*** devices. All reproducibility studies showed nearly total agreement with the exception of samples near the limit of detection, which is to be expected for qualitative tests.

Interfering Substances

Normal and TCC positive urine pools containing the substances listed below were tested in the ***Status BTA*** test.

TABLE VI. INTERFERING SUBSTANCES

SUBSTANCE	HIGHEST LEVEL TESTED WITH NO INTERFERENCE	LEVEL AT WHICH SUBSTANCE INTERFERED
Possible Urine Constituents		
Hemoglobin	100 mg/dL	No interference at MLT*
Red Blood Cells	10^6cells/mL	No interference at MLT
White Blood Cells	10^6cells/mL	No interference at MLT
Albumin	1 g/dL	No interference at MLT
Bilirubin(unconjugated)	0.4 mg/dL	0.8 mg/dL[A]
IgG	10 mg/dL	No interference at MLT
Uric Acid	250 mg/dL	No interference at MLT
Ascorbic Acid	5 g/dL	No interference at MLT
Caffeine	58.3 mg/dL	117 mg/dL[A]
Nicotine	14 mg/dL	28 mg/dL[A]
Sodium chloride	365 mg/dL	730 mg/dL[A]
Ethanol	1% (v/v)	No interference at MLT
Possible Micobial Contaminants		
Candida albicans	125 x 10^{10} CFU/mL	2.5 x 10^{10} CFU/mL[B]
Escherichia coli	2.5 x 10^{10} CFU/mL	No interference at MLT[C]
Pseudomonas aerugenosa	2.5 x 10^{12} CFU/mL	No interference at MLT[C]
Therapeutic Agents		
Ampicillin	600 mg/dL	No interference at MLT
Acetaminophen	520 mg/dL	5.2 g/dL[A]
Acetyl Salicylic Acid	520 mg/dL	5.2 g/dL[A]
Doxorubin-HCl	10 mg/dL	No interference at MLT
Mitomycin C	10 mg/dL	No interference at MLT
Nitrofurantoin	50 mg/dL	No interference at MLT
Phenazopyridine-HCl	80 mg/dL	100 mg/dL[A]
Thiotepa	10 mg/dL	No interference at MLT
Trimethoprim	50 mg/dL	No interference at MLT
Bacillus Calmette Guerin	20 mg/dL	No interference at MLT
Finasteride	2.5 mg/dL	No interference at MLT
Flutamide	100 mg/dL	No interference at MLT
Ioversol, 74% (imaging contrast agent)	1%	5%[A]
Urised	17.5 mg/dL	35 mg/dL[D]

* MLT - maximum level tested

[A] Negative Interference:substance decreased the intensity of a TCC positive urine test result

[B] 10 Subjecting samples to one freeze/thaw cycle resulted in no interference at 1.25 x 10^{10} CFU/mL, the MLT.

[C] Results of interference studies unchanged by subjecting samples to one freeze/thaw cycle

[D] Substance's coloration caused results for both normal and TCC positive urine to be difficult to interpret

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Symbols Key

	Instructions For Use (Read)
REF	Item Number
	Store At
	Expiration Date
CONT	Contents
IFU	Instructions For Use
PIP	Transfer Pipette
IVD	For In Vitro Diagnostic Use
LOT	Lot Number
	Manufacturer
MF	Manufactured For
EC REP	Authorized Representative
CE	CE Mark

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CE

EC REP

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