510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k062565

B. Purpose for Submission:

New device

C. Measurand:

Cotinine in urine

D. Type of Test:

Qualitative, visually read immunochromatographic assay

E. Applicant:

Princeton Biomeditech Corp.

F. Proprietary and Established Names:

Status DS Nicotine

AccuSign Nicotine

G. Regulatory Information:

1. Regulation section:

   21 CFR 862.3220, Carbon Monoxide test system

2. Classification:

   Class I, reserved

3. Product code:

   MKU, Enzyme Immunoassay, Nicotine and Nicotine Metabolites

4. Panel:

Toxicology (91)

H. Intended Use:

1. Intended use(s):

   See Indications for use.

2. Indication(s) for use:

   Status DS Nicotine

   Immunoassay for the qualitative detection of cotinine, a metabolite of nicotine, at the cut-off of 500 ng/mL in human urine. Status DS Nicotine is used as an aid in the detection of cotinine after use of tobacco products or other products containing nicotine. For In Vitro Diagnostic Use.

   The Status DS Nicotine test provides only a preliminary analytical result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography, mass spectrometry (GC/MS) is the preferred confirmatory method.

   AccuSign Nicotine

   Immunoassay for the qualitative detection of cotinine, a metabolite of nicotine, at the cut-off of 500 ng/mL in human urine. AccuSign DS Nicotine is used as an aid in the detection of cotinine after use of tobacco products or other products containing nicotine. For In Vitro Diagnostic Use.

   The AccuSign Nicotine test provides only a preliminary analytical result. A more specific alternative chemical method must be used in order to obtain a confirmed analytical result. Gas chromatography, mass spectrometry (GC/MS) is the preferred confirmatory method.

3. Special conditions for use statement(s):

   For prescription use.

4. Special instrument requirements:

   Not applicable

I. Device Description:

The device contains a membrane strip and a dye pad. The membrane strip is coated with monoclonal anti-cotinine antibody and the dye pad contains dye coated with

cotinine protein conjugate. A disposable urine dropper and instructions for use are included in the kit.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   OraSure Technologies Auto-Lyte Cotinine EIA

2. Predicate 510(k) number(s):

   k972481

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Test Type	Qualitative	Qualitative/Semi-quantitative
Cutoff	500 ng/mL	Same
Matrix	Urine	Same
Analyte	Cotinine	Same

Differences		
Item	Device	Predicate
Results	Visual	Instrument
Principle	Chromatographic Immunoassay	Enzyme Immunoassay

K. Standard/Guidance Document Referenced (if applicable):

None Referenced.

L. Test Principle:

The device uses a solid phase chromatographic membrane immunoassay technology and is based on the principle of specific immunochemical reactions between antigens and antibodies. The test relies on antibody binding competition between cotinine conjugate and the cotinine that may be present in the urine sample. In the test procedure, a sample of urine is placed in the sample well of the device and allowed to migrate up the membrane. If cotinine is present in the urine sample, it competes with the cotinine conjugate, which is bound to the dye, for the limited antibodies immobilized on the membrane. If the cotinine is present at or above the 500 ng/mL cutoff, cotinine will saturate the antibodies, thus inhibiting the binding of the dye coated with cotinine conjugate to the antibodies on the membrane. This prevents the formation of a line the membrane. A cotinine-positive urine sample will not generate

a line at the test position (T).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Qualitative imprecision was assessed by conducting a lot-to-lot precision study. The samples were prepared by adding cotinine to a known amount of drug-free specimen at concentrations 50% below the cutoff, 25% below the cutoff, 25% above the cutoff, 50% above the cutoff and 100% above the cutoff. The samples were tested with two lots of devices for two consecutive days. 10 samples for each of the 6 concentrations were tested daily for each lot.

The results are summarized in the table below:

Number of operators: 3
Number of lots: 2
Number of days: 2

Cotinine ng/mL	% Cutoff	Number Tested	Positive	Negative	% of Correct Results
0	0	120	0	120	100
250	50%	120	1	119	99
375	75%	120	19	101	84
625	125%	120	99	21	83
750	150%	120	117	3	98
1000	200%	120	120	0	100

A second qualitative imprecision study was conducted at three physician office laboratories using 15 blinded, randomized control samples: 5 negative samples, 5 samples at 50% below the cutoff and 5 samples at 100% above the cutoff. One operator performed the test at each site. The operators consisted of 2 nurses and one medical technician. There was 100% agreement at all concentration levels at all sites.

b. Linearity/assay reportable range:

Not applicable. The assay is intended for qualitative use.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

This device has internal process controls. A visible line appearing in the control region confirms that sufficient sample volume has been applied. In the labeling, users are instructed not to interpret the test if a visible line failed

to appear in the control region.

External control materials are not supplied with this device. External controls are available from commercial sources to ensure proper kit performance. In the labeling the sponsor recommends that the user run controls at regular intervals following federal, state and local guidelines.
Protocols and acceptance criteria were described for stability testing and found to be acceptable.

d. Detection limit:

The claimed cutoff for this assay is 500 ng/mL. The precision studies (above) demonstrate performance of the device around the cutoff. *e. Analytical specificity:*

The analytical specificity studies were performed on compounds structurally related to nicotine. All compounds were prepared in drug-free normal human urine. The following compounds produce positive results when tested at levels greater than the concentrations listed below.

Compound	Concentration (ng/ml)
Cotinine	500
Niacinamide	>100,000
(-) Nicotine	>100,000
Nicotinic Aid	>100,000
Nicotinic Acid N-oxide	>100,000
(±)-Anabasin	>100,000
(±)-Nornicotine	>100,000

Interference:
To evaluate the potential for interference by certain exogenous compounds, urine samples with concentrations at ± 25% of the cutoff were spiked with potential interferants and tested. No interferences were observed at the concentrations tested. Results are summarized below:

Substance	Concentration (mg/dL)
Ascorbic Acid	50
Protein	2000
Hemoglobin	100
Bilirubin	10
Glucose	1500
Creatinine	20

The table below lists compounds that showed no cross-reactivity when tested with the device. The interferents tested were at concentrations up to 100

µg/mL. A complete list is presented in the package insert.

Acetaminophen	Chlorpromazine	Doxylamine
Acetylsalicylate	Chlorquine	Ecgonine hydrochloride
Aminopyrine	Cholesterol	B-Estradiol
Amitryptiline	Chlomipramine	Ethyl-p-aminobenzoate
Amobarbital	Clonidine	Fenoprofen
Amoxicillin	Cocaine hydrochloride	Furoximide
Apomorphine	Codeine	Gentisic acid
Aspartame	Cortisone	Glutethimide
Atropine	Creatinine	Guaifenesin
Benzilic acid	Deoxycorticosterone	Hippuric Acid
Benzoic acid	Dextromethorphan	Ibuprofen
Butabarbital	Diazepam	(+/-)-Isoproterenol
Cannabidiol	Diclofenac	Ketamine
Chloralhydrate	Diethylpropion	Levorphanol
Chloramphenicol	Diflunsial	Lidocaine
Chlordiazepoxide	Digoxin	Methylphenidate
Chlorothiazide	Diphenhydrine	Naproxen
Dextromethorphan	Erythromycin	Oxalic acid
4-Dimethylaminoantipyrine	Ethanol	Penicillin-G
Dopamine	Glucose	Quinidine

Urinary pH studies:

Sample solutions containing drug concentrations that were 50% above and 50% below the cutoff were adjusted for pH between the ranges of 4.7 to 8. The pH adjusted sample solutions were tested ten times with the device. The results are summarized in the table below:

Cotinine Concentration (ng/mL)	pH	Number Tested	# Positive	# Negative	% Agreement
250	4.7	10	0	10	100
250	6.5	10	0	10	100
250	8.0	10	0	10	100
1000	4.7	10	10	0	100
1000	6.5	10	10	0	100
1000	8.0	10	10	0	100

No interferences due to pH were observed.

Urinary Specific Gravity studies:

Sample solutions containing drug concentrations that were 50% above and 50% below the cutoff were adjusted to specific gravities of 1.002 and 1.40 and tested ten times with the device. The results are summarized in the table below:

Cotinine Concentration (ng/mL)	Specific Gravity	Number Tested	# Positive	# Negative	% Agreement
250	1.002	10	0	10	100
250	1.040	10	0	10	100
1000	1.002	10	10	0	100
1000	1.040	10	10	0	100

No interferences due to different specific gravities were observed.

f. Assay cut-off:
Characterization of how the device performs analytically around the claimed cutoff concentration appears in the precision section above.

2. Comparison studies:

a. Method comparison with predicate and GC/MS:

The device was compared to the commercially available Auto-Lyte Cotinine EIA assay on seventy-nine unaltered clinical samples purchased from a laboratory (43 negatives and 36 positives). The results are summarized in the tables below:

Candidate device vs. Predicate device:

New device	Predicate		Total	% Agreement
Positive	36	0	36	100
Negative	0	43	43	100
Total	36	43	76	100

The device was compared to the reference method, Gas Chromatography/Mass Spectroscopy on eighty-six unaltered clinical samples purchased from a laboratory (39 negatives and 47 positives). The cotinine values ranged from 0 to 2155 ng/mL as measured by GC/MS. The results are summarized in the tables below:

Candidate device vs. GC/MS

	GC/MS				Total
New Device	Negative 0.75% Cutoff	Near Cutoff Negative (0.75% and cutoff)	Near Cutoff Positive (cutoff to 1.25%)	Positive (>1.25%)	
Positive	2*	5*	7	33	47
Negative	39	0	0	0	39

*The false positive samples contained cotinine values of 247, 360, 417, 427, 434, 437 and 470 ng/mL.

b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. Clinical Sensitivity:

Not applicable

b. Clinical specificity:

Not applicable

c. Other clinical supportive data (when a. and b. are not applicable):

Not applicable

4. Clinical cut-off:

Not applicable

5. Expected values/Reference range:

Not applicable

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.