CLSI PROCEDURE

Product Name: **Status Nicotine**
Item Number: 21735

Intuition:	
Prepared By:	Date:
Title:	

Accepted By:	Date:
Title:	

Accepted By:		Date:	

Discontinued By	Date:

CONTENTS

SECTION 1 - TEST NAME

Status Nicotine

CLIA Compexity: Moderate For Serum/Plasma; Moderate For Whole Blood

SECTION 2 - INTENDED USAGE

The **Status DS Nicotine** test is a simple, one-step, immuno-chromatographic
assay for the rapid, qualitative detection of cotinine, a major 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.*

SECTION 3 - SUMMARY AND EXPLANATION OF TEST

Smoking has been identified as a major risk factor for lung cancer and cardiovascular disease.1,2 Self-reporting of smoking status is not reliable.3 The detection of cotinine, a major metabolite of nicotine, has become the preferred biomedical method of assessing the smoking status of individuals on account of its sensitivity and specificity.4
Cotinine is present in blood, urine, and saliva of individuals who smoke or chew tobacco or who inhale tobacco smoke produced by others. As an objective indicator of nicotine intake or confirmation of nonsmoker status, cotinine offers several advantages over other biochemical measures: it is a specific indicator of nicotine intake, its concentrations are not influenced by confounding factors such as diet or environment, its average biological half-life in blood is 19 hours, and its concentration within a given individual varies by only 15 to 20% over the course of a day.5 Cotinine assay is thus

a superior objective measure of exposure to nicotine.

SECTION 4 - PRINCIPLE OF TEST

The **Status DS Nicotine** test uses solid-phase chromatographic membrane immunoassay technology for a qualitative detection of a nicotine metabolite, cotinine, in human urine.

The test is based on the principle of the highly specific immunochemical reactions between antigens and antibodies which are used for the analysis of specific substances in biological fluids. The test relies on the competition to bind to the antibodies between the cotinine conjugate and 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 is allowed to migrate upward. 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 cotinine level is above the cutoff level, 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 on the membrane. Therefore, a cotinine-positive urine sample will not generate a line at the Test position (T) in the Result window, indicating a positive result from positive cotinine competition, while a negative urine sample will generate a line at the Test position in the Result window, indicating a negative result from an absence of competition with free cotinine.

In addition to the Test line that may appear at the Test position (T), a Control line is present at the Control position (C) to confirm the viability of the test. This Control line (validation line) should always appear if the test is conducted properly. This works as a procedural control, confirming that proper sample volume was used and the reagent system at the control line and the conjugate-color indicator worked. If insufficient sample volume is used, there may not be a Control line, indicating the test is invalid.

SECTION 5 - KIT CONTENTS AND STORAGE

The **Status DS Nicotine** test kit contains all the reagents necessary to perform the assay.

• **Status DS Nicotine** device. The test 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.

• Disposable specimen dispenser.

• Instructions for use.

The **Status DS Nicotine** test kit should be stored at 2–30°C (35–86°F) in the original sealed pouch. The expiration dating given was established under these storage conditions.

SECTION 6 - MATERIALS REQUIRED BUT NOT PROVIDED

- Timer
- External positive and negative controls item #2100
- Latex gloves

SECTION 7 - WARNINGS AND PRECAUTIONS

• For *in vitro* diagnostic use only.

• Avoid cross contamination of urine samples by using a new urine specimen container and dropper for each urine sample.

• Urine specimens are potentially infectious. Proper handling and disposal methods should be established according to good laboratory practices.

• The **Status DS Nicotine** test device should remain in its original sealed pouch until ready for use. Do not use the test if the pouch is damaged or the seal is broken.

• Do not use the test kit after the expiration date.

SECTION 8 - PATIENT PREPARATIONS AND SPECIMEN COLLECTION

Approximately 110 µL of urine sample is required for each test. Fresh urine specimens do not require any special handling or pretreatment. Specimens should be collected in a clean glass or plastic container. If testing will not be performed immediately, specimens should be refrigerated (2–8°C) or frozen. The stability of specimens in a refrigerator or a freezer is established up to 5 weeks. Specimens should be brought to room temperature before testing.

Specimens containing a large amount of particulate matter may give inconsistent test results. Such specimens should be clarified by centrifuging or allowing settling before testing.

SECTION 9 - QUALITY CONTROL AND ASSURANCE

Internal Control: Each **Status DS** test device has built-in controls. The Control line is an internal positive procedural control. A distinct reddishpurple Control line should always appear at the C position, if the test procedure is performed properly, an adequate sample volume is used, the sample and reagent are wicking on the membrane, and the test reagents at the control line and the conjugate-color indicator are reactive. In addition, if the test has been performed correctly and the device is working properly, the background in the result window will become clear and provide a distinct result. This may be considered an internal negative procedural control.

If the Control line does not appear at the Control position, the test is invalid and a new test should be performed. If the problem persists, contact LifeSign for technical assistance.

External Control: External controls may also be used to assure that the reagents are working properly and that the assay procedure is followed correctly. It is recommended that a control be tested at regular intervals as good laboratory testing procedure and to follow federal, state, and local guidelines concerning the running of external quality controls for moderately complex testing.

SECTION 10 - TEST PROCEDURE

The **Status Nicotine** test procedure consists of adding the urine sample to the Sample well of the device and watching for the appearance of colored lines in the result window.

Test Protocol

1. For each test, open one **Status DS Nicotine** pouch and label the Status device with the patient ID. Lay the test device on a flat surface.

2. Holding the dropper vertically, dispense 3 full drops (110 µL) of the urine sample into the Sample well (S).

3. Read the result after 5 minutes, but within 10 minutes of sample application.

SECTION 11 - INTERPRETATION OF RESULTS

Negative: Two Lines. The appearance of two reddish-purple lines—one at the Test position (**T**) and the other at the Control position (**C**) in the Result window—indicates a negative test result; i.e., no cotinine above the cutoff level has been detected. The color of the Test line may be weaker or stronger than that of the Control line. *A negative test result does not indicate the absence of cotinine in the sample; it indicates only that the sample does not contain cotinine above the cutoff level in qualitative terms.*

Positive: One Line. The appearance of only one reddish-purple line at the Control position (C) in the Result window and no distinct line at the Test position (T) indicates the test result is positive (i.e., the specimen contains cotinine at a concentration above the cutoff level).

Invalid: A distinct colored line should always appear at the Control position (**C**). The test is invalid if no line forms in the Control position (**C**). *Note: A very faint line in the Test position (**T**), visible in 10 minutes, indicates*
that the amount of cotinine in the sample is near or below the cutoff level for the test.

SECTION 12 RESULT REPORTING

Status DS Nicotine is a qualitative assay. The amount of nicotine or cotinine (a nicotine metabolite) present in the urine cannot be estimated by the assay. The assay results distinguish positive from negative samples. Positive results indicate the samples contain cotinine above the cutoff concentration and the individual has been exposed to nicotine.

SECTION 13 LIMITATIONS

The test is designed for use with human urine only.

• There is a possibility that factors such as technical or procedural errors, as well as other substances not listed in the compounds tested in the urine sample, may interfere with the test and cause erroneous results.

• Adulterants, such as bleach and/or alum, in urine specimens may produce erroneous results. If adulteration is suspected, the test should be repeated with a new sample.

This test detects only the presence of cotinine in urine. A positive test result does not provide any indication of intoxication or urinary concentration.

• The test result read after 10 minutes may not be consistent with the original reading obtained within the 10 minute reading period. The test must be read within 10 minutes of sample application.

• Certain medications containing cotinine may produce a positive result in any chemical or immunological assay.

SECTION 14 PERFORMANCE CHARACTERISTICS

Refer to the Status Nicotine package insert for performance characteristics precision and interfering substances.

SECTION 15 REFERENCECES

1. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control: *Reducing the Health Consequences of Smoking, 25 Years of Progress, A Report of the Surgeon General*, Rockville, MD. Office on Smoking and Health, 1989.

2. U.S. Department of Health and Human Services, Public Health Service, *The Health Consequences of Smoking: Cardiovascular Disease, A Report of the Surgeon General*, Rockville, MD. Office on Smoking and Health, 1983.

3. Bruckert E, Jacob N, Lamaire L, Truffert J, Percheron F, and de Gennes JL. *Relationship between Smoking Status and Serum Lipid in a Hyperlipidemic Population and Analysis of Possible Confounding Factors*. Clin Chem 1992;38:1698-1705.

4. Pojer. R, Whitfield JB, Poulos V, Eckhard IF, Richmond R, Hensley WJ. *Carboxyhaemoglobin, Cotinine and Thiocyanate Assay Compared for Distinguishing Smokers from Non-smokers.* Clin Chem 1984;30:1377-1380.

5. Benowitz NL, Kuyt F, Jacob P, et al. *Cotinine Dispostion and Effects*. Clin Pharmacol Ther 34, 604-611 (1983).

SECTION 16 TECHNICAL ASSISTANCE

For technical assistance, contact LifeSign Technical Service Department at 1-800-526-2125

Corrective Action Form

Problem /Error	Corrective Action

Laboratory Technologist: _____ Date: _____

Laboratory Director: _____ Date: _____

Certification of Training

This is to verify that personnel responsible for running _____ test at

_____ have been thoroughly in-serviced on the test and the test procedure(s).

This has included:

Review of the package insert
Demonstration of the product assay
Successful performance of the test and interpretation of results

Names of the personnel who have been trained with the above test and are responsible for reporting patient results:

Print Name	Signature	Date

Signature(s) of those responsible for personnel and testing:

_____ _____
Signature Date

_____ _____
Signature Date

_____ _____
Signature Date

Testing Personnel Competency Assessment

Test _____

Procedure	Satisfactory	Unsatisfactory	Not Applicable	Comments/Corrective Action(s)
Observation of Test performance				
Patient Sample Preparation				
Specimen Handling/Processing				
Testing				
Recording/Reporting Results				
Assessment of Test Performance Using Known Samples				
Review of Records				
Patient/Quality Control Log Sheet Records				
Proficiency Testing Records				
Assessment of Problem Solving Skills				

(Attach all supporting documents)

Evaluator: _____ Date: _____

Testing Personnel: _____ Date: _____