Dextromethorphan
Diazepam
Diclofenac
Diethylpropion
Diflunisal
Digoxin
Diphenhydramine
Doxylamine
Ecgonine hydrochloride
ß-Estradiol
Ethyl-p-aminobenzoate
Fenoprofen
Furoxmide
Gentisic acid
Glutethimide
Guaifenesin
Hippuric acid
Hydrochlorothiazide
Hydrocodone
Hydrocortisone
Hydromorphone
3-Hydroxytyramine
O-Hydroxyhippuric acid
Ibuprofen
Imipramine
Iproniazid
(–) Isoproterenol
Isoxsuprine
Ketamine
Ketoprofen
Labetalol
Levorphanol
Lidocaine
Loperamide
Loxapine succinate
Maprotiline
Meperidine
Meprobamate
Methadone
Methaqualone
Methylphenidate
Methyprylon
Morphine-3-ß-D-glucuronide
Nalidixic acid
Nalorphine
Naloxone
Naproxen
Nifedipine
Norcodein
Norethindrone
Noroxymorphone
D-Norpropoxyphene
Noscapine
Nylidrin
D,L-Octopamine
Oxalic acid
Oxazepam
Oxolinic acid
Oxycodone
Oxymetazoline
Oxymorphone
Papaverine
Penicillin-G
Pentazocaine
Pentobarbital
Perphenazine
Phencyclidine
Phendimetrazine
Phenobarbital
Phenytoin
Phenylpropanolamine
Prednisolone
Prednisone
Promazine
Promethazine
D,L-Propanolol
Propiomazine
D-Propoxyphene
Quinidine
Quinine
Salicylic acid
Secobarbital
Serotonin
Sulfamethazine
Sulindac
Temazepam
Tetracycline
Tetrahydrocortisone
Ä⁹Tetrahydrocannabinol-carboxylic acid
Tetrahydrozoline
Thebaine
Thiamine
Thioridazine
D,L-Thyroxine
Tolbutamide
Triamterene
Trifluoperazine
Trimethoprim
Trimipramin

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Symbols Key

	Instructions For Use (Read)
REF	Item Number
2°C 30°C	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

Printed in U.S.A.
P-58113-E
644-6/16/17

CE

EC REP
MT Promedt Consulting GmbH
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MF Manufactured for:
lifeSign
A PBM Group Company
85 Orchard Road,
Skillman, NJ 08558
800-526-2125, 732-246-3366
www.lifesignmed.com

P-58113-E

Status DS Nicotine

One-Step Nicotine Test

For Laboratory *In Vitro* Use Only

Simple One-Step Immunoassay for the Qualitative Detection of Nicotine Metabolite in Urine

CLIA Complexity: Moderate

Stock No.	21735	35 Test Kit
	21710	10 Test Kit

Intended Use

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.*

Summary and Explanation

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.

Principle

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.

Materials Provided

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.

Materials Needed but Not Provided

- Timer
- External positive and negative controls

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** 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.

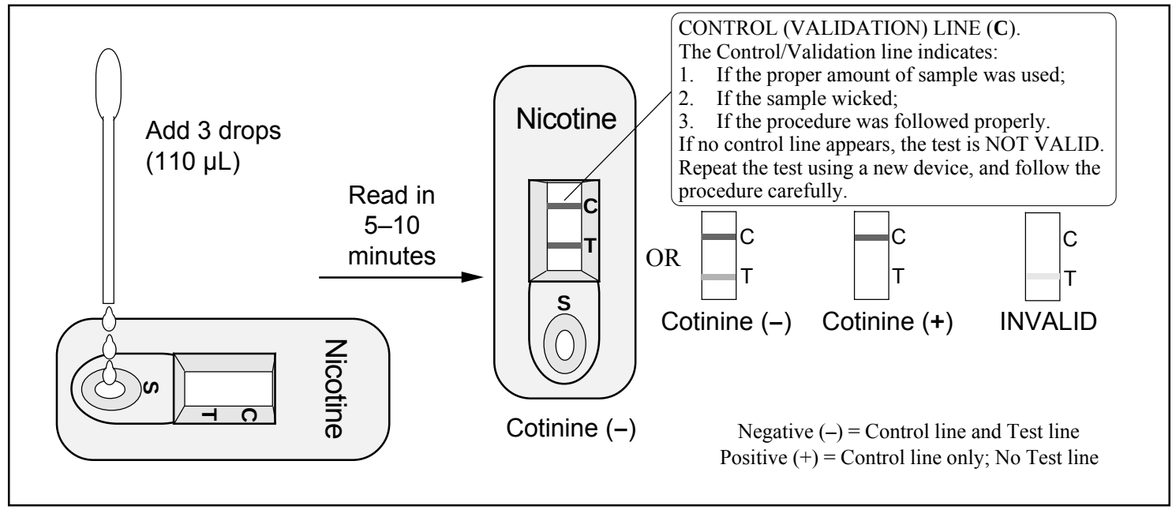
Storage and Stability

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.

Specimen Collection and Preparation

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.



Test Procedure

The 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.
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.

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.*

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.

User Quality Control

Internal Control: Each **Status DS** test device has built-in controls. The Control line is an internal positive procedural control. A distinct reddish-purple 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 information on how to obtain controls, contact LifeSign's Technical Services.

Expected Values

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.

Performance Characteristics

The performance of **Status DS Nicotine** was compared with commercially available Cotinine EIA test. The complete agreement (>99%) was observed. Results are shown in the Table 1.

Table 1. Status DS Nicotine test vs an EIA test

Status Nicotine	Auto-Lyte Cotinine EIA		Total
	Positive	Negative	
Positive	36	0	36
Negative	0	43	43
Total	36	43	79

In a separate study, the accuracy of **Status DS Nicotine** test was evaluated in comparison to the cotinine value of the sample as determined by the GC/MS. The samples contained cotinine from 0 ng/mL to 2155 ng/mL by GC/MS. The result is shown in the table 2.

Table 2. Correlation with GC/MS value

Status DS Nicotine	GC/MS Values				Total
	Negative		Positive		
	A	B	C	D	
Positive	2	5	7	33	47
Negative	39	0	0	0	39
Total	46		40		86

A: Negative (75 % cutoff)

B: Near Cutoff Negative (between 75 % and cutoff)

C: Near Cutoff Positive (between cutoff and 125%)

D: Positive (greater than 125 % cutoff)

All false positives samples contained cotinine greater than 350 ng/mL and possibly due to the cross-reactivity of nicotine's metabolites. Total agreement was 92%.

Precision

The precision of the **Status DS Nicotine** test was determined by carrying out the test with serially diluted standard drug solutions (Cotinine). Three people carried out the study. The prepared concentrations were equal to 50% below cutoff, 25% below cutoff, cutoff, 25% above cutoff, 50% above cutoff and 100% above cutoff. There were no significant differences between operators, between days or between lots. Table 3 shows the precision data that were combined all operators' tests.

Table 3. Precision Data

Cotinine conc. ng/mL	# Tested	# Positive	# Negative	% Correct Results
0	120	0	120	100
250	120	1	119	99
375	120	19	101	84
625	120	99	21	83
750	120	117	3	98
1000	120	120	0	100

Reproducibility

The reproducibility of the test results of the **Status DS Nicotine** test was examined at three different sites using a total of 15 blind controls, consisting of 5 negative samples, 5 at half cutoff level (250 ng/mL cotinine), and 5 positive samples (1,000 ng/mL cotinine). The results obtained at these three sites with these controls demonstrated 100% agreement with expected results.

Specificity

The specificity of **Status DS Nicotine** test was determined by adding the compounds structurally related to nicotine to cotinine-negative urine specimens and testing with the **Status DS Nicotine** test kit. The results are expressed in terms of the concentration required to produce a positive result (Table 4).

Table 4. Specificity

Compound	Concentration (ng/mL)
Cotinine	500
Niacinamide	>100,000
(-)-Nicotine	>100,000
Nicotinic acid	>100,000
Nicotinic acid N-oxide	>100,000
(±)-Anabasine	>100,000
(±)-Nornicotine	15,000

Interfering Substances

Endogenous Compounds

Status DS Nicotine test showed no interference when the endogenous compounds were added at the concentration given below (Table 5) to urine samples which had +25% cutoff concentration of cotinine.

Table 5. Endogenous compounds

Substance Added	Concentration added
Albumin	2000 mg/dL
Hemoglobin	100 mg/dL
Bilirubin	10 mg/dL
Glucose	1500 mg/dL
Creatinine	20 mg/dL

Exogenous Compounds

The following compounds show no cross-reactivity when tested with **Status DS Nicotine** at a concentration of 100 µg/mL (Table 6).

Table 6. Non Cross-Reacting Compounds

Acetaminophen	Atropine	Chlorpromazine
N-Acetylprocainamide	Benzilic acid	Chlorquine
Acetylsalicylate	Benzoic acid	Cholesterol
Aminopyrine	Benzoylecgonine	Clomipramine
Amitryptyline	Benzphetamine	Clonidine
Amobarbital	Butabarbital	Cocaine hydrochloride
Amoxicillin	Cannabidiol	Codeine
Apomorphine	Chloralhydrate	Cortisone
Aspartame	Chloramphenicol	Creatinine
Ascorbic acid	Chlordiazepoxide	Deoxycorticosterone
	Chlorothiazide	