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| <b>Procedures:</b> Status Nicotine |
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| Prepared by | Date Adopted | Supersedes Procedure # |
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**PRINCIPLE:**

The Status™ Nicotine test uses solid-phase chromatographic membrane immunoassay technology for the qualitative, simultaneous 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 in the test window, indicating a positive result from positive cotinine competition, while a negative urine sample will generate a line in the test window, indicating a negative result from an absence of competition with free cotinine.

In addition to the Test line that may appear in the Test window (T), a Control line is present in the Control window (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.

**SPECIMEN:**

- 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. 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 to settle before testing.

**Storage Requirements:**

The **Status™ 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.

**PROCEDURE - STEPWISE:**

1. For each test, open one **Status™ 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 5–10 minutes.

**REPORTING RESULTS:**

**Negative:** Two Lines. The appearance of two reddish-purple lines—one in the Test window (**T**) and the other in the Control window (**C**)—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 in the Control window (**C**) and no distinct line in the Test window (**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 in the Control window (**C**). The test is invalid if no line forms in the Control window (**C**).

*Note: A very faint line in the Test window (**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 OF THE PROCEDURE:**

- 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 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 regardless of the method of analysis. 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.

### **QUALITY CONTROL:**

**Internal Control:** Each Status™ test device has a built-in control. The Control line is an internal process positive control. A distinct reddish-purple Control line should always appear in the 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 working. 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 process control.

The positive and negative process controls contained in each Status®™est device satisfy the requirements of testing a positive control and a negative control on a daily basis. If the Control line does not appear in the Control window, 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 and assay procedure are performing properly. It is recommended that a control be tested at regular intervals as good laboratory testing practice. For information on how to obtain controls, contact LifeSign's Technical Services.

### **REFERENCES:**

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 I, 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 Disposition and Effects*. Clin Pharmacol Ther 34, 604-611 (1983).