

COTININE TEST

C-101

One Step Test for the Detection of Cotinine in Human Urine.

Intended Use

The cotinine test is a lateral flow, one-step immunochromatographic assay for the qualitative detection of cotinine in human urine. The test obtains qualitative results and is intended for the determination of smoking status only. This assay is a screening test. A more specific testing method must be used in order to obtain a confirmed analytical result. Clinical consideration and professional judgment should be applied to all test results, particularly when preliminary positive results are indicated.

Summary

Tobacco smoking results in the absorption of nicotine through the lung and nasal epithelium, after which nicotine is metabolized into about 20 metabolites excreted in urine. Cotinine, a major metabolite, accumulates in the body with regular smoking. The test is a one step simple and easy immunoassay for the qualitative detection of cotinine in human urine. It is based on the principle of highly specific immunochemical reactions of antigens and antibodies.

Principle

The cotinine test contains a membrane strip that is pre-coated with cotinine antigen at the test line region. The cotinine antibody gold conjugate pad is placed at the end of the membrane. In cotinine-free urine, the colored antibody-colloidal gold conjugate and urine move chromatographically by capillary action across the membrane. This solution migrates to the test line containing cotinine antigen and forms a visible line as the antibody complexes with the antigen. The formation of a visible precipitant in the test zone indicates a negative result (non-smoker). When cotinine is present in urine, it will fill the limited antibody binding sites. This will prevent attachment of the colored antibody-colloidal gold conjugate at the test line region. Therefore, absence of the color band on the test region indicates a positive result (smoker).

A different antigen-antibody reaction is added to the membrane strip at the control region (C) to indicate that the test has been performed properly. This control line should always appear, regardless of the cotinine status in the urine. This means that negative urine will have two pink colored bands, and positive urine will have only one pink colored band. The pink colored control band serves as an indicator that 1) sufficient volume has been added, and 2) that proper flow was obtained.

Storage and Stability

The test kit should be stored refrigerated or at room temperature 2 – 30°C. Each device should remain in its sealed pouch for the duration of the shelf-life or until use.

Precautions

Urine specimens may be potentially infectious and should be handled according to good laboratory practice. Avoid cross-contamination of urine samples by using a new specimen collection container and specimen dropper for each urine sample.

Materials provided

Test devices, each in a hermetically sealed foil wrapper, containing a desiccant and a dropper. The wrapper should be opened only when the test is ready to be used.

Specimen Collection and Handling

According to good Laboratory Practice, handle all samples as if capable of transmitting Hepatitis and HIV. Avoid contact with skin by wearing gloves and proper laboratory attire.

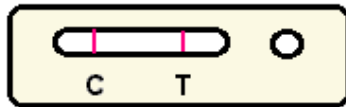
The urine samples should be collected in clean dry plastic or glass containers. Fresh urine does not require any special handling or pre-treatment. Test should be performed soon after the urine specimen is collected, preferably the same day. The specimen may be refrigerated at 2 – 30°C for 3 days or frozen at –20°C for a longer period of time. Specimens that have been refrigerated must be brought to room temperature prior to testing. Specimens previously frozen must be thawed, equilibrated to room temperature, and mixed thoroughly prior to testing.

Test Procedure

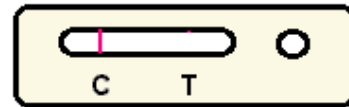
Test device, patient samples, and controls should be brought to room temperature (20 – 30 °C) prior to testing. Do not open pouches until ready to perform the assay. Use a new test device and a new dropper for each test.

1. Tear the foil wrapper and remove the test device (bring the device to room temperature before opening the pouch to avoid condensation of moisture on the membrane). Place on a flat surface with the openings facing upward. Label the devices with patient or control identification.
2. Fill the dropper with urine.
3. Hold the dropper vertically and add 3 drops of sample, approximately 100 µl (without air bubbles), into the small, circular well.
4. Read result between 3 to 8 minutes after the addition of samples. Do not interpret results after 8 minutes.

Interpretation of Results



Negative: Both the test line (T) and the control line (C) should appear in the long window. The control line (C) indicates proper performance of the device. The test line intensity may be weaker or stronger than that of the control line.



Positive: Only one colored line appears in the control line region (C). No colored line appears in the test line region (T).

INVALID: No colored line appears in the control region. If the control line (C) does not appear, the test result is inconclusive and the assay should be repeated.

NOTE: A very faint line in the test region indicates that the cotinine concentration in urine is near the cut-off level for the test. These samples should be re-tested or confirmed with a more specific method before a positive result is determined.

Limitation of Procedure

The assay is designed for use with human urine only.

A positive result indicates only that the presence of cotinine is above the cut-off concentration. It does not indicate or measure level of consumption.

There is a possibility those technical or procedural errors as well as other substances or factors not listed may interfere with the test and cause false results. If it is suspected that the samples have been mislabeled or deteriorated, a new specimen should be collected and the test should be repeated.

Quality Control

A built-in control is included in the test. In addition, according to good laboratory practice, positive and negative controls should be used with each assay. Such controls are available from commercial sources.

Sensitivity

The Cotinine test will detect 200 ng/ml or more of cotinine. The accuracy of the test was evaluated in comparison to a commercially available immunoassay at a cut off concentration of 200ng/ml with 100% agreement.

Specificity

The specificity for the Cotinine test was tested by adding various drugs, drug metabolites, and other compounds that are likely to be present in urine. All compounds were prepared in cotinine-free normal human urine.

References

1. Zevin S., Jacob P., and Benowitz N. Cotinine effects on nicotine metabolism. *Clinical Pharmacology & Therapeutics*, June 1997 61(6):649-54.
2. Zuccaro P., Pichini S., Atieri I., Rosa M., Pellegrini M., and Pacifici R., Interference of nicotine metabolites in cotinine determination by RIA. *Clinical Chemistry*, Jan 1997 43(1): 180-1.
3. Benowitz NL. Cotinine as biomarker of environmental tobacco smoke exposure. *Epidemiologic Reviews*, 1996 18(2):188-204.
4. Curvall M., and Vala FK., Nicotine and metabolites: analysis and levels in body fluids. *Nicotine and related alkaloids: absorption, distribution, metabolism, and excretion*. Edited by Gorrod JW and Wahren J. Published in 1993 by Chapman & Hall, London.