accutest® NicAlert™ Urine

NEVER PLACE NICALERT™ STRIP IN MOUTH

INTENDED USE

NicAlert™ is intended for in vitro diagnostic professional use for the semi-quantitative determination of cotinine in urine for the purpose of determining if an individual has been exposed to tobacco products such as cigarettes, pipes, or chewing tobacco within the past 48 hours. The cutoff concentration for the NicAlert™ test is 100 ng/mL. Second hand smoke exposure (environmental tobacco smoke) may cause a positive result in a non-user of tobacco products. The NicAlert™ Positive and Negative Controls are intended for in vitro diagnostic use for the quality control of the NicAlert™ test.

BACKGROUND

The knowledge and awareness of the health hazards associated with exposure to tobacco products, especially smoking cigarettes, is well established.[1-8] Cigarette smoking has been identified as one of the most significant causes of death and disease in the U.S. (Surgeon General's Report of the U.S. Public Health Service, Year 2000). Smoking has been cited as being responsible for 87% of deaths from lung cancer, 21% of deaths from coronary heart disease, 18% of deaths from stroke, and 82% of deaths from chronic obstructive pulmonary disease. Significantly elevated risks of disease and death are also associated with other forms of tobacco use such as pipe and cigar smoking and the use of chewing tobacco.

As an adjunct to self-reporting of smoking behavior, and as a more objective approach, the assay of biochemical markers is of established importance. Urinary nicotine is not a reliable indicator of smoking status as it has a comparatively short half-life.[9] Cotinine is a major metabolite of nicotine and it has a relatively long half-life (10-40 hours). Cotinine has been shown to be more sensitive and specific than CO monitoring for measuring smoking status.[9-13] The reference method used for measuring cotinine is Gas Chromatography/Mass Spectrometry (GC/MS)[14], or Liquid Chromatography / Mass Spectrometry (LC/MS/MS)[15].

PRINCIPLE OF THE TEST

NicAlert™ is an immunochromatographic assay that uses monoclonal antibody-coated gold particles and a series of avidity traps that allow quantification. It employs patented technologies, (U.S. Patent Nos. 5,527,686; 5,710,009; 6,087,185 and 6,121,008). The sample collection end of the strip contains gold particles coated with monoclonal antibodies to cotinine, a relatively long-lived metabolite of nicotine. The distance the gold migrates on the strip is shown by a clear color change and provides an accurate measure of the amount of cotinine in the sample.

MATERIALS PROVIDED

1. NicAlert™ Strip: Each NicAlert™ test strip is individually packaged in a sealed labeled plastic pouch.
   Each test strip is composed of the following:

   a) 5mm X 90mm nitrocellulose impregnated with:
   b) Mouse monoclonal antibodies reactive to cotinine, conjugated to colloidal gold particles.
   c) Rabbit anti-mouse polyclonal antibodies reactive to mouse antibodies.
   d) 1% Cobalt Blue 406 #2 dye.
   e) Carboxycotinine, isopropyl carboxynorcotinine, carboxyphenylethylcotinine bromide.
   f) 50mM sodium phosphate buffer pH 7.2, bulking agents, stabilizers.
   g) Cotton pads, filters.

2. Controls:

   a. NicAlert™ Negative Control (cotinine 0 ng/mL)
   b. NicAlert™ Low Positive Control (cotinine 400 ng/mL)
   c. NicAlert™ High Positive Control (cotinine 2000 ng/mL)

   The NicAlert™ Positive and Negative Controls are human urine-based liquid and are ready to use. These Controls each contain a known concentration of cotinine (Negative: 0 ng/mL; Low Positive: 400 ng/mL; High Positive: 2000 ng/mL). The NicAlert™ Positive Control is prepared by spiking known concentrations of cotinine into the NicAlert™ Negative Control, which is preserved human urine with no detectable amount of cotinine by LC/MS/MS. Negative Control human urine is tested negative for human pathogens (including HBV, HCV, HIV). The nominal concentrations of cotinine in the NicAlert™ Controls are determined and confirmed by LC/MS/MS. See vial labels for expiration date and for opened and closed vial stability.

Materials Required But Not Provided:

A timer or clock.
For urine sample collection a clean, leak-proof, disposable container (urine specimen container) is required.
For urine samples, latex or rubber gloves are needed for urine collection, and forceps, tweezers or gloves are preferred for holding the strip when dipping it into the urine sample.

QUALITY CONTROL

Good laboratory practice recommends periodic use of quality control procedures. It is recommended that the NicAlert™ Human Urine Positive and Negative Test Controls be run at least once per day when clinical specimens are tested. The use of controls from other commercial vendors is also recommended. Users should follow the appropriate federal, state and local guidelines concerning the running of external quality controls. The NicAlert™ Positive and Negative Test Controls are intended for in vitro diagnostic use for the quality control of the NicAlert™ test. The NicAlert™ Negative Controls consist of cotinine-free (NicAlert™ Level "0") human preserved pathogen-free urine. The NicAlert™ Positive Controls consists of cotinine-free urine spiked with cotinine to concentrations of 400 ng/mL cotinine (Low Positive Control, NicAlert™ level "4") and 2000 ng/mL cotinine (High Positive Control, NicAlert™ level "6").

Quantity	Product Description
500 µL	NicAlert™ Negative Control - Preserved human urine tested to be negative for cotinine, verified by LC/MS/MS.
500 µL	NicAlert™ Low Positive Control - Preserved human urine containing cotinine 400 ng/mL ± 10%, verified by LC/MS/MS.
500 µL	NicAlert™ High Positive Control - Preserved human urine containing 2000 ng/mL ± 10%, verified by LC/MS/MS.

STORAGE

Store NicAlert™ at room temperature, out of direct sunlight, in the sealed pouches. The test strips can be used up until the expiration date indicated on the label. Once the package is opened, the strip should be used within 10 minutes. NicAlert™ Positive and Negative Controls should be stored at 2°-8°C. After opening, do not use the Controls if the contents become cloudy or altered in appearance.

WARNINGS AND PRECAUTIONS

1. Do not put any part of the NicAlert™ strip in your mouth.
2. Treat samples as a potential biohazard and discard appropriately after testing.
3. Use the NicAlert™ strip within 10 minutes of opening the pouch.
4. Discard any samples if contamination is suspected and obtain another sample.
5. Do not consume or handle food or drink near the NicAlert™ strip or when performing the test.
6. Test samples at room temperature.
7. The NicAlert™ strip should not be used on cloudy or pink urine samples.

WARNING: NEVER PLACE A NICALERT™ STRIP IN YOUR MOUTH

TESTING URINE SAMPLES WITH NICALERT™

Before Starting

Before starting, you will need:

- a NicAlert™ strip in its pouch with a valid expiry date;
- clean, leak-proof, disposable container for the urine sample;
- a watch, timer, or clock; and;
- a clean nonabsorbent surface (such as a countertop or a plastic surface) on which to place the NicAlert™ strip (do not use absorbent materials such as paper towels, etc.).

You may also prefer to use:

- latex or rubber gloves for urine collection;
- forceps, tweezers or gloves to hold the strip when dipping it into the urine sample.

COLLECTING AND STORING THE URINE SAMPLE

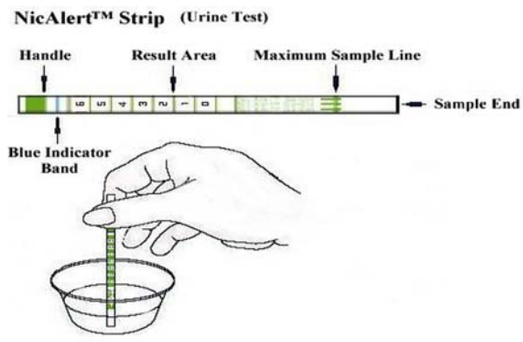
Caution: Handle any urine sample as if it was a potential biohazard. Discard appropriately after testing.

1. Use a clean leak-proof, disposable container such as a urine specimen container. Label the container if more than one person is being tested at a time. Do not re-use containers. Dispose of containers after use.
2. Collect approximately 25 mL (1 fluid oz.) of urine. The person providing the sample should wash his or her hands both before and after providing the sample. The person should collect a mid-stream sample by collecting the sample after a few seconds of the start of urination. If needed, use latex or rubber gloves.
3. Visually check the urine sample for signs of contamination (foreign material such as bathroom tissue or hair, cloudiness, particles, blood, etc.). Do not use the sample if contamination is suspected and obtain another sample instead.
4. Test the sample within 4 hours of collection. Urine samples can be stored for up to 3 days if refrigerated at 39º F (4º C) immediately after collection or stored indefinitely if frozen at -4º F (-20º C) immediately after collection. Use a screw top tube or similar sealed container for storage. Allow refrigerated or frozen samples to reach room temperature before testing.

OPENING THE SEALED NICALERT™ PLASTIC POUCH

1. Open the NicAlert™ pouch by tearing at the slit on the side.
2. Remove the NicAlert™ strip and lay the strip on a flat dry surface.
3. Use the NicAlert™ strip within 10 minutes of opening the pouch.

TESTING THE URINE SAMPLE WITH NICALERT™



1. Holding strip by the handle, dip the sample end (the exposed soft cotton end of the strip) into the urine sample to a depth of 1/2 inch (1 cm) holding the strip with forceps, tweezers or gloves if preferred. Do not immerse the strip lower than the maximum sample line on the strip.
2. Hold the strip in the urine for twenty seconds. Do not over-saturate.
3. Remove the strip and lay it flat on a nonabsorbent surface to develop for approximately 10 to 15 minutes until the blue indicator band (Figure 2) substantially fades or disappears. During this period, you should see at least one reddish band appear in the numbered white zones in the strip.

HOW TO READ AND INTERPRET NICALERT™ RESULTS FOR URINE SAMPLES

5. A reddish color must appear in at least one of the numbered zones (Levels 0 – 6) on the strip and there must not be any noticeable color in the area between the sample end (the exposed soft cotton end of the strip) and Level 0. Otherwise the results are not valid and the test must be repeated with a new NicAlert™ strip. If the color appears as an indistinct smear throughout the strip, the results are not valid and the test must be repeated with a new NicAlert™ strip.

6. Find the lowest numbered zone (Level 0 – 6) on the strip with a reddish color in it. This lowest level is the NicAlert™ test result. Note: The strip may have more than one level in which there is a reddish band or color. Different levels may have different shades or colors in them. The presence of a reddish color in the lowest numbered level is the test result (see Figure 2).

7. The NicAlert™ test result is the lowest numbered level on the strip with a reddish band or color in it. If there is doubt as to the lowest level, express the result as a range of 2 levels, eg. "1-2" or "5-6," etc., or repeat the test.

8. For urine samples, a NicAlert™ result of:
   - Level 3 or higher (Level 4, 5, or 6) indicates use of tobacco products;
   - Level 0 indicates no detectable level of cotinine or tobacco product use;
   - Levels 1 (10-30 ng/mL) and Level 2 (30-100 ng/mL) indicate no use of tobacco products.
   - A borderline result at the cutoff (i.e. NicAlert™ level "2-3") could be a smoker or a non-smoker. Repeat testing with another urine sample is recommended.

EXPRESSING THE NICALERT™ TEST RESULTS AS COTININE CONCENTRATION RANGES

FIGURE 2: READING THE NICALERT™ RESULT

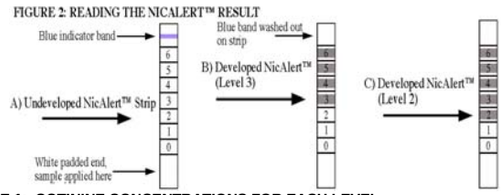


TABLE 1: COTININE CONCENTRATIONS FOR EACH LEVEL

Level	Cotinine Concentration (ng/mL)	Interpretation (see "Clinical Studies" below)
0	1-10	Non-user of tobacco products
1	10-30	Non-user of tobacco products
2	30-100 (Below the cutoff, negative)	Non-user of tobacco products
3	100-200 (Above the cutoff, positive)	User of tobacco products
4	200-500	User of tobacco products
5	500-1000	User of tobacco products
6	>1000	User of tobacco products

LIMITATIONS OF THE PROCEDURE

NicAlert™ is only for use with human urine. A positive result indicates tobacco product exposure and the presence of cotinine in the sample. Erroneous results can be caused by technical or procedural errors or by adulteration or contamination of the sample. NicAlert™ should not be used if the urine is dark, red, or otherwise abnormally colored. The test should be delayed until the urine is clear and normal in appearance. The assay provides only a preliminary result. Clinical consideration and professional judgment must be applied to any test result, particularly in evaluating a preliminary positive result. In order to obtain a confirmed analytical result, a more specific alternate chemical method is needed. Gas Chromatography/Mass Spectrometry (GC/MS) is the preferred confirmation method.

PERFORMANCE CHARACTERISTICS

Stability

Stability testing has demonstrated that NicAlert™ strips have a shelf life of at least two years when stored at ambient temperatures.

Limit of Detection

The lower limit of detection of the NicAlert™ test was determined by the addition of various amounts of cotinine (50 ng/mL, 25 ng/mL, 15 ng/mL, 10 ng/mL, 5 ng/mL, confirmed by mass spectrometry) to aliquots of human urine obtained from non users of tobacco products (spiked negative ("0") urine). Results from three lots in duplicate were read by three trained individuals. The lowest cotinine concentration at which a clearly discernable reading ("1") was visible in 100% of test runs was 10 ng/mL.

Accuracy and Cutoff

Urine samples (N = 174) from smokers (N = 119) and non-smokers (N = 55) were collected from patients at 4 point of care (POC) sites (medical clinics). Individuals were classified as: A) Smokers and other tobacco product users (such as users of chewing tobacco) and, B) Non-smokers, on the basis of self-reporting by questionnaire. The inclusion criteria for smokers were: any individual who self reported to have consumed any amount and any type of tobacco product (cigarettes, cigars, or chewing tobacco) within 48 hours prior to collection of the sample. The inclusion criteria for non-smokers were: any individual who self reported not to have consumed any tobacco products within 2 months prior to collection of the sample. Exposure to second hand smoke was not considered during enrollment. The samples were tested at 4 sites by a total of 7 different non-laboratory individuals (2, 2, 2, and 1 individuals per site). Urine samples were assayed for cotinine by liquid chromatography/mass spectrometry (LC/MS/MS) (N = 167), and by gas chromatography. The distribution of NicAlert™ results compared to LC/MS/MS (N = 167) according to smoker versus non-smoker is summarized in Table 2:

TABLE 2: ACCURACY STUDY RESULTS

A. Non-Smokers

Cotinine Concentration by LC/MS/MS (ng/mL)	Number of Samples	NicAlert™ Reading of "0"	NicAlert™ Reading of "1"	NicAlert™ Reading of "2"
Not detected	30	24	6	0
1-10	13	6	6	1
11-30	6	2	2	2
31-100	1	0	0	1

B. Smokers

Cotinine Concentration by LC/MS/MS	Number of Samples	NicAlert™ Reading of "0"	NicAlert™ Reading of "1"	NicAlert™ Reading of "2"	NicAlert™ Reading of "3"	NicAlert™ Reading of "4"	NicAlert™ Reading of "5"	NicAlert™ Reading of "6"
Not Detected	2	2	0	0	0	0	0	0
1-10	4	3	1	0	0	0	0	0
11-30	6	0	2	3	1	0	0	0
31-99	8	0	0	7	0	0	1	0
100-200	12	0	2	0	6	1	2	1
201-500	14	0	0	0	1	3	7	3
501-1000	20	0	0	0	0	0	5	15
1001-2500	41	0	0	0	0	1	2	38
>2500	10	0	0	0	0	0	2	8

Cross Reactions

NicAlert™ detects cotinine, a metabolite of nicotine, in urine. The following structurally-related compounds were tested for cross-reactivity at concentrations up to and including 100,000 ng/mL.

Compound	Concentration Tested, ng/mL	% Cross Reactivity
Niacinamide	100,000	n.d.
Nicotine	100,000	n.d.
Nicotinic Acid	100,000	n.d.
Nicotinic Acid N-Oxide	100,000	n.d.

n.d. = not detected

3-OH cotinine is a known cross-reactant with cotinine in immunoassays. 3-OH cotinine was spiked into cotinine negative ("0" NicAlert™) urine at the following concentrations: 50 ng/mL, 150 ng/mL, 250 ng/mL, 750 ng/mL, and 1200 ng/mL. 3-OH cotinine showed a 12-40% cross-reactivity with cotinine in the NicAlert™ assay.

Interference Studies

The following additional compounds were added to normal human urine negative for cotinine ("0" NicAlert™), and tested for cross-reactivity at concentrations of 10,000 ng/mL and 100,000 ng/mL. The added compounds were also tested for cross-reactivity at the above concentrations (10,000 and 100,000 ng/mL) when added to urine spiked with cotinine at the following concentrations: 50 ng/mL (NicAlert™ "2," negative), 150 ng/mL (NicAlert™ "3," positive), and 1500 ng/mL (NicAlert™ "6," positive). Additional compounds tested for cross-reactivity included: aspirin, acetaminophen (tylenol), ibuprofen, penicillin, caffeine, niacin, pheniramine, brompheniramine, chloropheniramine. The results were compared to controls obtained for unadulterated, spiked normal human urine. The substance was considered not to interfere if there was no change in the NicAlert™ reading as compared to the control materials. Using these criteria, none of the substances were found to have a positive or a negative effect on the NicAlert™ readings at the levels tested. The effects of other interfering substances and variables were examined in the NicAlert™ test. Ascorbic acid, pH, specific gravity, bacteria, protein, hemoglobin, bilirubin, and glucose were spiked into urine containing 0, 100, 750, and 1500 ng/mL cotinine. The results were compared to controls obtained for unadulterated, spiked normal human urine. The substance or variable was considered not to interfere if there was no change in the NicAlert™ reading as compared to the control materials. Using these criteria, none of the substances or variables were found to have a positive or negative effect on the NicAlert™ readings.

Interferent	Levels Tested
Ascorbic Acid	10, 20, 50 mg/dL
pH	4, 5, 7, 8
Specific Gravity	1.000, 1.015, 1.030
Protein	25, 100, 250 mg/dL
Hemoglobin	0.3, 0.1 mg/dL
Bilirubin	5 mg/dL
Glucose	10, 100, 500 mg/dL
Bacteria	(-) and (++)

It is possible that other substances and / or factors not listed above may interfere with the test and cause false results, e.g., technical or procedural errors.

Reproducibility Studies

At each of three clinical sites, assay precision near the cutoff level was assessed by examining the performance of the NicAlert™ test in duplicate samples on four separate occasions from each of two lots of 8 urine specimens provided by Nymox, consisting of spiked urines near the cutoff level (50 ng/mL (NicAlert™ "2") , 75 ng/mL (NicAlert™ "2"), 125 ng/mL (NicAlert™ "3"), 150 ng/mL, (NicAlert™ "3")).

At three sites, a total of four untrained non-laboratory individuals (consisting of nurses and paramedical workers) performed the NicAlert™ test under blinded random code to establish assay precision near the cutoff. The operators read 100% of the 125 ng/mL samples as NicAlert™ "3" (positive, above the cutoff) and 92.8% of the 50 ng/mL samples as NicAlert™ "2" (negative, below the cutoff), indicating an overall assay precision near the cutoff (50 ng/mL (NicAlert™ "2", below the cutoff) and 125 ng/mL (NicAlert™ "3", above the cutoff) of 96.4%. (Data summarized in Table 3, below).

Cotinine free urine (NicAlert™ Level "0") samples were spiked with known amounts of cotinine. All samples were confirmed by mass spectrometry (LC/MS/MS). Table 3 includes precision and recovery studies from readings performed by a total of 8 individuals on a total of 551 samples.

TABLE 3: REPRODUCIBILITY STUDIES

Concentration	Number of Positives (NicAlert™ level reading of 3 or higher)	Number of Negatives (NicAlert™ level reading of 2 or lower)
0 ng/mL *	0	60
20 ng/mL *	0	60
50 ng/mL *	9	114
125 ng/mL *	128	0
350 ng/mL *	60	0
750 ng/ml *	60	0
1200 ng/mL *	60	0

* These studies were performed by Nymox.

* * These concentrations were performed by POC personnel.

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