



NicAlert™ Saliva

Product Insert

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 saliva 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 10 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. 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

NicAlert™ Strip: Each NicAlert™ test strip is individually packaged in a sealed labeled plastic pouch.
(REF 836070003388)

Funnel
Saliva Tube container
Snap on top with specialized filter

Each test strip is composed of the following:

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

OPTIONAL MATERIALS

Controls:

- NicAlert™ Negative Control (cotinine 0 ng/mL)
- NicAlert™ Low Positive Control (cotinine 400 ng/mL)
- NicAlert™ High Positive Control (cotinine 2000 ng/mL)

The NicAlert™ Positive and Negative Controls are synthetic saliva-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 synthetic saliva with no detectable amount of cotinine 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.

QUALITY CONTROL

Good laboratory practice recommends periodic use of quality control procedures. The use of controls from other commercial vendors is also recommended. Users should follow the applicable regulatory 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") synthetic saliva. The NicAlert™ Positive Controls consist of cotinine-free synthetic saliva 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 - Synthetic saliva tested to be negative for cotinine, verified by LC/MS/MS. |
| 500 µL | NicAlert™ Low Positive Control - Synthetic saliva containing cotinine 400 ng/mL ± 10%, verified by LC/MS/MS. |
| 500 µL | NicAlert™ High Positive Control - Synthetic saliva 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

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

WARNING: NEVER PLACE A NICALERT™ STRIP IN YOUR MOUTH

TESTING SALIVA SAMPLES WITH NICALERT™

Before Starting

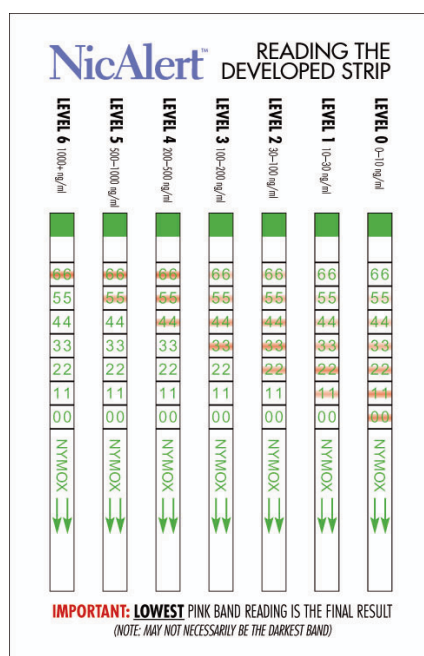
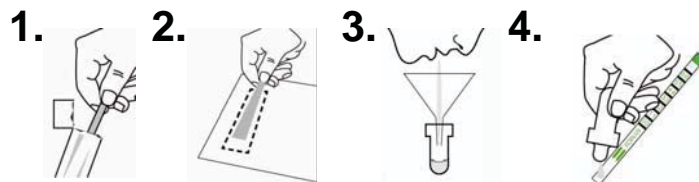
Before starting, you will need:

- a NicAlert™ strip in its pouch with a valid expiry date;
- a funnel, cap and collection vial
- 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.). Alternately the laminated instruction card can be used.

PROCEDURE

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

- Open the NicAlert™ package (1.) and lay the NicAlert™ strip down flat on a non-absorbent surface (do NOT put the NicAlert™ on paper or Kleenex). The plastic laminated instruction card has an area marked on it for this purpose. (2.)
- Tear open the NicAlert™ Saliva Collection Kit and remove the funnel, the saliva tube container, and the snap-on top for the saliva tube container.
- Place the funnel in the saliva tube container and spit/deposit saliva into the funnel, enough to fill at least 1/2 of the saliva tube container. (3.) Then discard the funnel.
- Snap on the top of the saliva tube container. Squeeze 8 drops from the inverted saliva tube directly onto the white padded end of the NicAlert™ strip. (4.)



Reading the SalivaNicAlert™:

- Read the NicAlert™ after 15 to 30 minutes when the blue band has disappeared or faded substantially. Bands may darken over this period. Bands should not disappear so lower readings such as a 0 may be obtained quickly.
- The **LOWEST band is the result.** A reddish band must appear in at least one of the numbered zones (Levels 0 - 6) on the strip.

Note: The marking levels on the strip appear as a 66 ("6"), 55 ("5"), etc. This is in order to make the strip easier to read.

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.

Note: The strip often has 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 band in the lowest numbered Level is the test result. It does NOT have to be the darkest band.

EXPRESSING THE NICALERT™ TEST RESULTS AS COTININE CONCENTRATION RANGES

TABLE 1: COTININE CONCENTRATIONS FOR EACH LEVEL

| Level | Cotinine Concentration (ng/mL) | Interpretation (see "Clinical Studies" below) |
|-------|--------------------------------|---|
| 0 | 0-10 | Non-user of tobacco products |
| 1 | 10-30 | User of tobacco products |
| 2 | 30-100 | User of tobacco products |
| 3 | 100-200 | 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™ saliva is for use with human saliva. 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™ saliva should not be used if the saliva appears to be abnormal 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

Saliva controls were diluted to 20,10,8,6,4,2 and 0 ng/mL cotinine and read in duplicate with Nicalert Strips.

| Concentration in ng/mL | 20 ng/mL | 10 ng/mL | 8 ng/mL | 6 ng/mL | 4 ng/mL | 2 ng/mL | 0 ng/mL |
|------------------------|----------|----------|---------|---------|---------|---------|---------|
| NA Reading | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| NA Reading | 1 | 1 | 0 | 0 | 0 | 0 | 0 |

The Limit of Detection is 10 ng/mL.

Accuracy and Cutoff:

Based on smoking status as determined by urine cotinine measurement by GC-MS (50 ng/mL cut-off), the saliva strip test results, averaged over the two operators, had a sensitivity of 99% and a specificity of 96%.

Subjects:

The participants were 172 consecutive individuals seen as primary care outpatients at 2 sites. 5 patients (2.9%) were excluded from the final analysis: in 3 cases, there was an inadequate amount of saliva provided by the subject, and in 2 cases the operator was unable to report a reading from the strip (presumed to be due to technique or mishandling). Of the analyzed group (n = 167) 36.5% (n = 61) were men and 62.3% (n = 104) women; for two subjects sex was not noted. The mean age of subjects with recorded age was 42.1 years with the youngest subject being age 6 and the oldest 80. The overall prevalence of current self-reported smoking in the group was 46.1% (n = 77). The characteristics of the study population are summarized in Table 1.

Table 1: GC-MS Determination of Smoking Status (50 ng/mL cut-off) vs. saliva NicAlert™ determination for the 2 Operators at each Site

| GC-MS | Site 1 | | | |
|-------------------------|----------------------------|-----------------------|----------------------------|-----------------------|
| | Operator 1 | | Operator 2 | |
| | Non-Smoker (NicAlert™ = 0) | Smoker (NicAlert™ ≥1) | Non-Smoker (NicAlert™ = 0) | Smoker (NicAlert™ ≥1) |
| Non-Smoker (< 50 ng/mL) | 47 | 2 | 45 | 4 |
| Smoker (≥ 50 ng/mL) | 0 | 32 | 1 | 29 |
| Sensitivity | 100% | | 96.7% | |
| Specificity | 95.9% | | 91.8% | |
| GC-MS | Site 2 | | | |
| | Operator 1 | | Operator 2 | |
| | Non-Smoker (NicAlert™ = 0) | Smoker (NicAlert™ ≥1) | Non-Smoker (NicAlert™ = 0) | Smoker (NicAlert™ ≥1) |
| Non-Smoker (< 50 ng/mL) | 32 | 0 | 31 | 1 |
| Smoker (≥ 50 ng/mL) | 0 | 47 | 0 | 44 |
| Sensitivity | 100% | | 100% | |
| Specificity | 100% | | 96.7% | |

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Confirm Biosciences Inc
 10123 Carroll Canyon Road
 San Diego, CA 92131
 800-908-5603
 info@confirmbiosciences.com
 www.confirmbiosciences.com

Cross-Reactants

Negative Saliva was spiked with compounds related to cotinine at 100,000 ng/mL
 Nicotinic Acid - Hazy no distinct bands
 Nicotinic Acid n-oxide - no crossreactivity
 Niacinamide - no crossreactivity
 Nicotine - Trap Level 3

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.

Interfering Substances

Saliva Standards (0 ng/mL, 60 ng/mL, 150 ng/mL and 2000 ng/mL) were spiked with:
 Hemoglobin - No Interference
 Protein - No interference at (2.5 mg/mL)
 pH 4.0 - Level 1 trap seen at all cotinine concentrations 2,3,4,5,6 read as 1
 pH 5.0 - Level 3 reads as 1, Level 5 reads as 3, Level 6 reads as 4
 pH 6.0 - Level 3 reads as 2, Level 5 reads as 4 Level 6 reads as 4
 pH 7.0 and pH 8.0 ran normally
 Ascorbic Acid Spiked at 0.5 mg/mL - Traps 0,1,2,3 Normal, Traps 4,5,6 reading lowered by one
 Ethanol 10% solution - Traps 0,1,2,3 Normal, Traps 4,5,6 reading lowered by one
 Pheniramine - No effect
 Aspirin - No effect
 Caffeine - No effect
 Penicillin - No effect
 Ibuprofen - No effect
 Acetaminophen - No effect
 Chlorpheniramine - No effect
 Brompheniramine - No effect

Reproducibility

- Three saliva control levels were tested in duplicate by three readers over three days.

| Reader | 0 ng/mL | 150 ng/mL | 2000 ng/mL |
|----------------|---------|-----------|------------|
| Tester 1 Day 1 | 0, 0 | 3, 3 | 6, 6 |
| Tester 2 Day 1 | 0, 0 | 3, 3 | 6, 6 |
| Tester 3 Day 1 | 0, 0 | 3, 3 | 6, 6 |
| Tester 1 Day 2 | 0, 0 | 3, 3 | 6, 6 |
| Tester 2 Day 2 | 0, 0 | 3, 3 | 6, 6 |
| Tester 3 Day 2 | 0, 0 | 3, 3 | 6, 6 |
| Tester 1 Day 3 | 0, 0 | 3, 3 | 6, 6 |
| Tester 2 Day 3 | 0, 0 | 3, 3 | 6, 6 |
| Tester 3 Day 3 | 0, 0 | 3, 3 | 6, 6 |

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