

NicAlert™

THE NicAlert™ STRIP MUST NEVER BE PLACED DIRECTLY IN THE MOUTH

INTENDED USE

NicAlert™ is designed to determine smoker status and detect the presence of tobacco products consumed by other means. Examples of its use include determining whether a teenager or student athlete is smoking, whether an insurance applicant qualifies for a non-smoking rate or whether a research subject in a smoking cessation study has successfully stopped smoking. NicAlert™ is not designed for medical diagnosis or therapeutic purposes.

BACKGROUND

The knowledge and awareness of the health hazards associated with exposure to tobacco products, especially smoking cigarettes, is well established and publicized. Indeed cigarette smoke has been identified as one of the most significant causes of death and disease in the United States. (Surgeon General's Report of the U.S. Public Health Service for the year 2000). Smoking has been identified as the cause of death in 87% of cases of lung cancer, 21% of cases of coronary disease, 18% of strokes and 82% of cases of chronic obstructive lung disease (1, 2). Alternative forms of consumption, such as pipe and cigar smoking or chewing tobacco, are also associated with a significantly increased risk of disease or death (3, 4). As a complement to self-reporting on smoking habits, the use of tests based on biochemical markers is proving to be practical and is therefore on the increase. Earlier methods involved using specific tests to measure levels of carbon monoxide (CO) and the metabolites of nicotine. The levels of CO occurring naturally in the body are relatively low, and increase considerably if tobacco is inhaled. The amount of nicotine in urine is not a reliable indicator of smoker status, as nicotine has a short half-life period and is quickly metabolised by the circulatory system. Of the major metabolites, cotinine is a suitable candidate for a marker as it has a relatively long half-life of 10-40h and has been shown to be more sensitive and specific than CO monitoring for measuring smoking status (5). The reference method used for measuring cotinine is gas chromatography/mass spectrometry (GC/MS).

TEST PRINCIPLE

NicAlert™ is an immunoassay, which uses gold particles coated with monoclonal antibodies and a range of "avidity traps" to allow the quantity to be determined.

Monoclonal antibodies to cotinine are coated on gold particles, which are then deposited on the sample application pad. Any cotinine in the sample binds to the antibody on the gold particle. When the cotinine binds to the particle, it occupies a binding site. The strength of a particle's ability to bind to a trap is a function of the number of unoccupied binding sites; the more available the binding sites, the greater the ability to bind. Each bound cotinine thus decreases the ability of the particle to bind, and one can use a very weak binding partner in trap one to trap particles that have no bound cotinine. Successive traps of increasing avidity for the particles can trap the particles that are not bound in trap one. The number of occupied binding sites is a function of the amount of cotinine, so the distance the gold migrates is directly related to the amount of cotinine in the sample.

ITEMS SUPPLIED

Each NicAlert™ Strip Test Kit contains NicAlert™ strips sealed in individual foil pouches. The strip can be used with either urine or saliva. When testing saliva, you must use the saliva collection kit.

NicAlert™ Saliva Collection Kits Each collection kit includes: - Funnels for saliva deposit - 2ml tube containers for collection of saliva samples - Snap-on tops for the saliva tube containers

Items required, but not provided: Gloves or forceps for handling the strip after it has been used. OPTIONAL ITEMS: Supermint®, Tictac®, or other white mint-flavoured sweet for stimulating saliva flow.

WARNINGS AND PRECAUTIONS

***DO NOT INGEST OR PUT ANY PART OF THE NicAlert™ IN THE MOUTH.**

The consumption or handling of food or drink near the NicAlert™, or when the test is being performed, is NOT recommended.

Samples should be tested at room temperature.

Sample adulteration may give a false result. If adulteration is suspected, obtain another sample and repeat the test.

Use the NicAlert™ within 10 minutes of opening the foil pouch.

If the subject has a dry mouth you may wish to stimulate saliva flow using a Supermint®, Tictac®, or other white mint-flavoured sweet. Part of the dissolved mint may be spat into the tube along with the saliva, but the test must be carried out immediately.

STORAGE

NicAlert™ test strips should be stored at room temperature, out of direct sunlight and in their sealed foil pouches. The strips can be used until the expiration date indicated on the package label. If the foil pouch is opened, the strip must be used within 10 minutes.

SAMPLE COLLECTION

Saliva should be obtained and tested within 4 hours of sample collection. Saliva can be stored for up to 3 days if refrigerated to 4°C immediately after collection, or frozen immediately to –20 °C if a longer period of storage is required. Stored samples should be warmed to room temperature before testing. Do not use the specimen if adulteration or contamination is suspected. **Do not use exudate from the sinuses as a saliva source.**

Urine should be tested within 4 hours of being obtained. Urine can be stored for up to 3 days if refrigerated to 4°C immediately after collection, or frozen immediately to –20 °C if a longer period of storage is required. Stored samples should be warmed to room temperature before testing. Do not use the specimen if adulteration or contamination is suspected.

Saliva and urine samples should be handled as if potentially infectious and as biohazards.

TEST PROCEDURE FOR SALIVA:

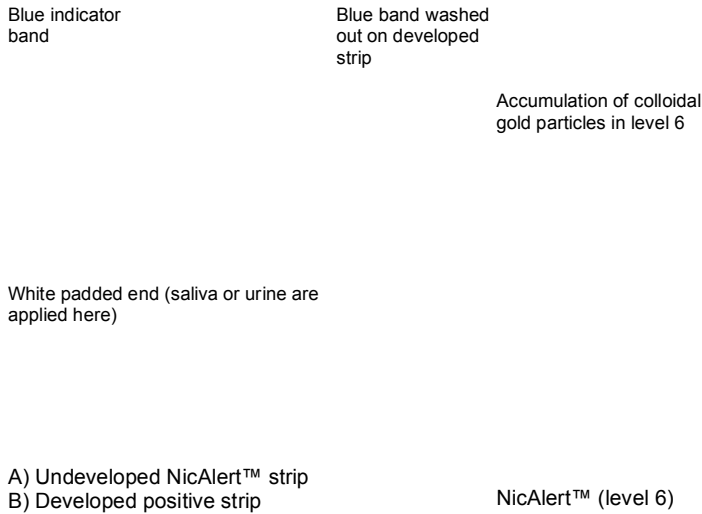
1. Place the funnel in the tube. Deposit saliva in the funnel and collect a sufficient amount to fill at least a third of the tube.
2. Discard the funnel and place a sealing cap on the tube, applying pressure until it snaps into place.
3. Open a pouch containing a NicAlert™ strip by tearing at the slit on the side and lay the strip on a flat surface.
4. Invert the closed tube. Slowly squeeze eight drops of saliva from the tube directly onto the white padded end of the strip. (The green section is at the opposite end of the strip).
5. Leave the strip on a flat surface until the red area moves up into the white area above it. Wait for bands to appear. Read the strips once the blue band at the end of the strip has virtually disappeared. If the blue band has not disappeared after 20 minutes, apply several more drops of sample and incubate for an additional 20 minutes (some saliva samples are very viscous and require a longer time to migrate upwards).

6. A red colouration must appear in at least one of the zones (Levels 0-6). Otherwise the test results are not valid. **If the blue band does not disappear after additional incubation, or if the readout looks smeared rather than appearing as series of distinctive bands, the sample being used is too viscous. Do not retest with the same sample.** Repeat the procedure with the Supermint® or Tictac®, etc. and test this sample with a new NicAlert strip.

TEST PROCEDURE FOR URINE:

1. The test may be done by dipping or by “wicking” (as described below).
2. Collect the urine in any clean container, e.g. a 2oz. beaker.
3. **DIPPING** - Holding the strip with gloves or forceps by the green end, dip the soft cotton end of the strip into the urine to a depth of 1.5 cm (not more) and hold for five seconds. Remove the strip and place it on a flat surface for 10-15 minutes, or until the blue test band disappears. **WICKING** – Pour ¼” urine into any small container, to a depth of not more than 1.5cm and holding the strip with gloves or forceps, immerse the strip and leave it to stand. The strip may be removed once the blue test band at the top disappears. The strip will not over-develop.
4. A red colour must appear in at least one of the zones (Levels 0-6). Otherwise the results are not valid, and the test must be repeated.

Fig. 1: Interpreting the NicAlert™ results



RESULTS AND INTERPRETATION

The result is based on the area with the least amount of colouration. The level is expressed as a value from 0 to 6. See fig. 2 for examples of how the results should be interpreted.

Fig. 2: Examples showing interpretation of NicAlert™ results

Least colouration in level 6 Reading value = level 6

Least colouration in level 1 Reading value = level 1

INTERPRETATION

Level 0 = least colouration at level 0 = enter as level 0. Level 1 = least colouration at level 1 = enter as level 1. Level 2 = least colouration at level 2 = enter as level 2, and so on.

A NicAlert™ reading of **level 3 (100-200ng/mL)** or higher in urine indicates the use of tobacco products: see table 2a

A NicAlert™ reading of **level 1 (10-30 ng/ml)** or higher in saliva indicates the use of tobacco products: see table 2b

The NicAlert™ test strip may be read at any time after completion of the test. If stored away from sunlight, the strip can provide a stable record for three years or more.

The results are expressed as “levels”: NicAlert™ is designed in such a way that each level corresponds to a concentration range for cotinine and/or 3-hydroxycotinine (see table 1).

Table 1. Cotinine equivalents for each level:	
Level	Cotinine equivalents (ng/ml)
0	1 – 10
1	10 – 30
2	30 – 100
3	100 – 200
4	200 – 500
5	500 – 2000
6	> 2000

The level should be expressed in terms of “cotinine equivalents” for the purposes of assigning a semi-quantitative measure of tobacco exposure, as the test detects both cotinine and hydroxycotinine. Cotinine equivalents are the approximate concentrations of cotinine and hydroxycotinine in the sample.

Table 2a: Comparison of the Sensitivity and Specificity of NicAlert™, GC/MS, and STC Elisa and STC Autolyte assays in urine (with cutoff values)

Sensitivity				
Number of tobacco users	NicAlert™	GC/MS	STC Elisa	STC Auto Lyte®
133	100 ng/ml	50 ng/ml	500 ng/ml	500 ng/ml
	87 %	83 %	68 %	70 %
Specificity				
Number of verbal non-users				
56	100 %	100 %	100 %	100 %

Table 2b: Comparison of the sensitivity and specificity of NicAlert™ with STC Elisa in saliva (with cutoff values)

Sensitivity				
Number of tobacco users	NicAlert™	GC/MS	STC Elisa	STC Elisa
102	10 ng/ml	10 ng/ml	20 ng/ml	30 ng/ml
	75 %	75 %	68 %	63 %
Specificity				
Number of verbal non-users				
46	100 %	100 %	100 %	100 %

LIMITATIONS OF THE PROCEDURE

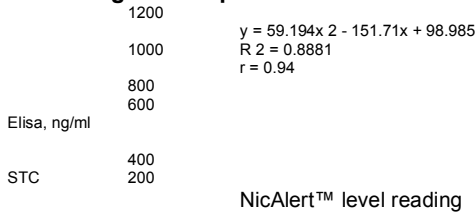
The NicAlert™ test is not intended for medical diagnostic or therapeutic purposes. A positive result indicates only exposure to tobacco products and the presence of cotinine and hydroxycotinine in the sample. Erroneous results can be caused by technical or procedural errors, or by adulteration or contamination of the sample.

PERFORMANCE CHARACTERISTICS

Comparison of NicAlert™ with the STC Elisa assay

A set of 150 saliva samples was field-tested with NicAlert™ and sent to a laboratory for analysis with STC Cotinine Elisa. A cutoff level of 10 ng/ml was used for determining the degree of exposure to tobacco products. The correlation between NicAlert™ and the Elisa assay resulted in $r = 0.94$. See fig. 3 and tables 2a and 2b.

Fig. 3: Comparison of saliva cotinine levels determined using NicAlert™ and STC Elisa



Accuracy

Variation within a strip

A negative batch of saliva was brought to levels of 60, 300 and 2100 ng/ml by having cotinine added. Each batch was tested ten times with NicAlert™. All ten replicates of the 60 ng/ml cotinine mixture produced a level 2 (30-100 ng/ml) reading. All ten replicates of the 300 ng/ml cotinine mixture produced a level 4 (200-500 ng/ml) reading. All ten replicates of the 2100 ng/ml cotinine mixture produced a level 6 (> 2000 ng/ml) reading.

Day-to-day variation

A batch of saliva from non-smokers, known to be negative for cotinine, was spiked with cotinine to produce levels of 60, 300 and 2100 ng/ml. Each batch was tested with NicAlert™ ten times, on ten separate occasions and over a ten-day period. The 60 ng/ml cotinine mixture produced level 2 (30-100 ng/ml) readings on all ten occasions over the ten-day period. In same way and over the same period, all ten tests on the 300 ng/ml cotinine mixture and the 2100 ng/ml cotinine mixture produced level 4 (200- 500 ng/ml) and level 6 (>2000 ng/ml) readings respectively.

Interference 1.

pH

Partial amounts from a negative batch (with pH 7.2) were adjusted with 1M of lemon juice to pH 4, 4.5, 5 and 6 respectively. In addition, 0.5 M of sodium carbonate was used to adjust partial amounts of saliva to pH 8, 9, 9.5 and 10. Cotinine was then added to all saliva samples to bring them to a level of 150 ng/ml. All the treated samples were tested immediately with NicAlert™. The results are shown in table 3.

Table 3: The effect of pH on NicAlert™

pH	NicAlert™ readings	
	Expected	Actual
4	3	3
4,5	3	3
5	3	3
6	3	3
7,2	3	3
8	3	3
9	3	3
9,5	3	3
10	3	4

The NicAlert™ test produced, as expected, readings of between pH 4 and 9.5. The normal pH range for saliva is 6.5 to 6.9, and it is known to have a large buffer capacity (7), i.e. it has a strong tendency to resist changes in pH.

2. Cross-reactants

Hydroxycotinine: Saliva from a negative batch was added to hydroxycotinine to adjust its level to 0.125, 0.25, 0.5, 1, and 2 µg/ml. All solutions were then tested with NicAlert™. All levels of hydroxycotinine gave a cross-reactivity reading of ~25%.

Nicotine: Saliva from a negative batch was added to nicotine to adjust its level to 2, 10 and 20 µg/ml. All solutions were then tested with NicAlert™. The untreated and 2 µg/ml mixtures produced level 1 readings, while the 10 and 20 µg/ml mixtures showed level 2, indicating maximum cross-reactivity of <1 %.

The following additional pyridine derivatives were added to a negative batch of saliva to adjust to a level of 50 µg/ml: niacinamide, nicotinic acid (niacin), nicotinic hydrazide, isonicotinic hydrazide, iproniazide phosphate, metyrapone, isonicotinic acid. All of these solutions were tested with NicAlert™ and produced a level 0 reading, which is regarded as a negative result.

3. Other sources of interference

Chlorpheniramine added to a negative saliva batch to adjust to a level of 200 µg/ml did not affect the assay. The test produced a level 0 reading, which is regarded as a negative result. Glucose, ascorbic acid, albumin, and haemoglobin were added to adjust to 0 µg/ml (control) and mixed with either 500 µg/ml or 50 µg/ml of a saliva batch adjusted to a cotinine level of 120 ng/ml (level 3) and tested with NicAlert™ (table 4). All of these compounds produced a level 3 reading, which indicates that there was no interference from these substances that could affect the cotinine readings.

Table 4: Effects of glucose, albumin, haemoglobin and ascorbic acid on NicAlert™

Substance added	Mixture level, in µg/ml	NicAlert™ readings (120 ng/ml cotinine)	
		Expected	Actual
Glucose	0	3	3
	500	3	3
Ascorbic acid	0	3	3
	500	3	3
Albumin	0	3	3
	50	3	3
Haemoglobin	0	3	3
	50	3	3

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