

Accutest® NicAlert™

NEVER PLACE NicAlert STRIP IN MOUTH

INTENDED USE

Accutest® NicAlert™ is intended for use for determining smoking status or other forms of tobacco product use. 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. Accutest® NicAlert™ is not intended for medical diagnostic 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. In fact 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^(1,2). 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^(3,4).

As an adjunct to self-reporting of smoking behavior, the assay of biochemical markers is a viable and increasingly used method. Historically, carbon monoxide (CO) monitoring and assays specific for nicotine metabolites have been used. CO levels found naturally in the body are relatively low and they rise considerably upon inhalation of tobacco smoke. Urinary nicotine is not a reliable indicator of smoking status as it has a short half-life and is rapidly metabolized in 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).

PRINCIPLE OF THE TEST

Accutest® NicAlert™ is an immunoassay that utilizes monoclonal antibody coated gold particles and a series of avidity "traps" that allow quantitation.

Monoclonal antibodies to cotinine are coated on gold particles that are 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. Thus, each bound cotinine 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 and thus the distance the gold migrates is directly related to the amount of cotinine in the sample.

MATERIALS AVAILABLE

1. Accutest® NicAlert™ Strip Test Kit contains 50 NicAlert™ strips sealed in individual foil pouches. The strip can be used for either urine or saliva. When testing saliva, you must use the saliva collection kit.

2. Accutest® NicAlert™ Saliva Collection Kits. Each collection kit includes:

- 50 funnels for saliva deposit
- 50 x 2ml tube containers for collection of saliva sample
- 50 snap on tops for the saliva tube container

MATERIALS REQUIRED BUT NOT PROVIDED: Gloves or forceps for handling the strip after it has been run.

OPTIONAL Materials: Supermint®, tictac®, or other white mint for stimulating saliva flow.

WARNINGS AND PRECAUTIONS

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

*The consumption and handling of food and 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 client has a dry mouth you may wish to stimulate saliva flow using a Supermint® or tictac®, or other white mint. The mint may be spit into the tube along with the saliva and the test must be run immediately.

STORAGE

The Accutest® NicAlert™ test strips should be stored at room temperature, out of direct sunlight in the 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.

SPECIMEN COLLECTION

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

Saliva should be obtained and tested within 4 hours of sample collection. Saliva can be stored for up to 3 days if refrigerated at 4 °C immediately after collection or frozen immediately at -20 °C if a longer period of storage is required. Stored specimens 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.**

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

ASSAY PROCEDURE SALIVA:

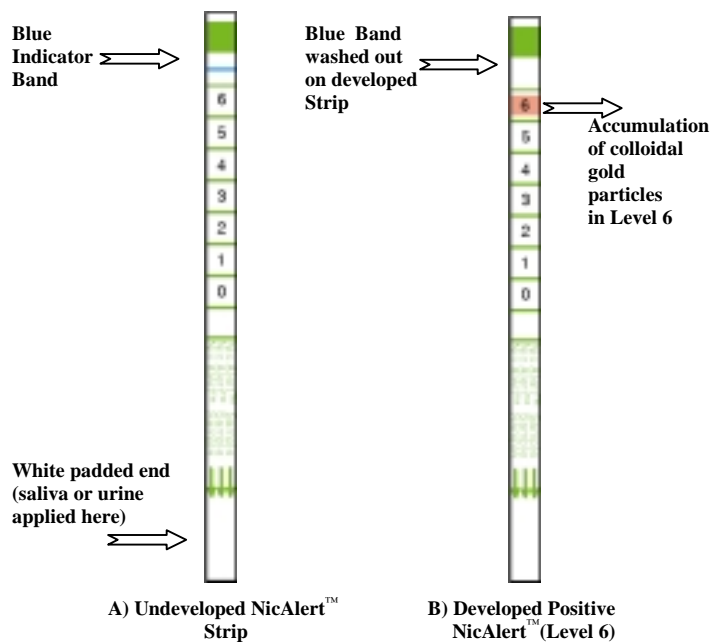
1. Place funnel in tube. Deposit saliva into funnel and collect a sufficient amount of sample to fill at least 1/3rd of tube.
2. Discard funnel and apply tube top applying pressure until it snaps into place.
3. Open a pouch containing an Accutest® NicAlert™ strip by tearing at the slit on the side and lay the strip on a flat surface.
4. Invert closed tube. Slowly squeeze 8 drops of saliva sample from tube directly on the exposed white padded end. (The green end is at the opposite end of the strip).
5. Leave strip to rest on a flat surface until the red area transfers up into the white area above it. Wait for bands to develop. Read the strips once the blue band at the end of the strip substantially disappears. If the blue band has not disappeared after 20 minutes, apply several more drops of sample and incubate for an additional 20 minutes (some salivas are very viscous and require a longer time to migrate upwards).
6. A red color 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 does not appear as discreet bands but a smear, the sample being used is too viscous. Do not retest this sample. Collect another sample using a Supermint® or tictac®, etc. and rerun the sample on a new Accutest® NicAlert™ strip.**

ASSAY PROCEDURE URINE:

1. The test may be done by Dipping or by Wicking (described below)
2. Collect urine in any clean container, e.g. a 2-oz. Dixie cup.
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 1/2 inch (not more) and hold for five seconds. Remove the strip and lay flat for 10-15 minutes until the blue test band disappears.
WICKING - Pour ¼" urine in any small container, to a depth of not more than ½ inch and holding the strip with gloves or forceps, insert strip and leave. Once the blue test band disappears at top of strip, the strip may be removed. The strip will not over develop.

A red color must appear in at least one of the zones (Levels 0-6). Otherwise the results are not valid and the test must be repeated.

Figure 1: Reading the Accutest® NicAlert™ Result

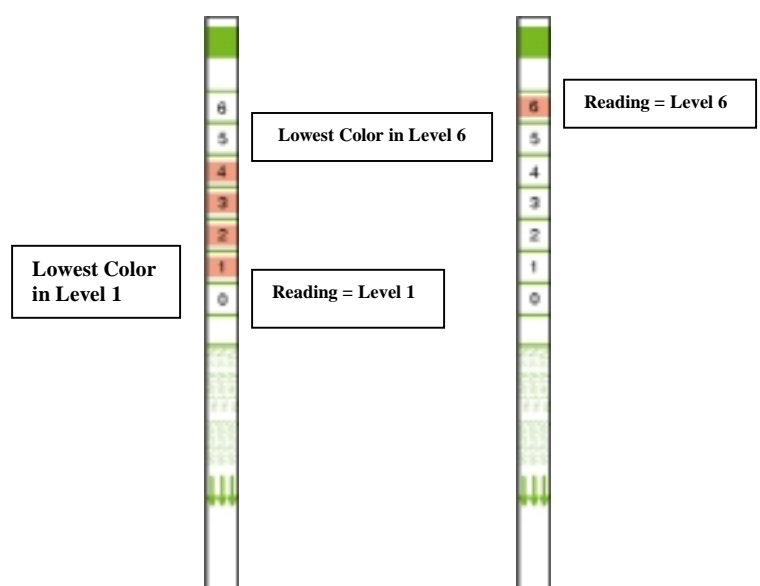


RESULTS AND INTERPRETATION

1. READ THE RESULTS AS THE LOWEST ZONE WITH COLOR.

Record the Level as 0-6. See figure 2 for examples of how to read the results.

Figure 2: Examples showing reading of NicAlert™ results.



INTERPRETATION

- Level 0 = Lowest color at Level 0 = Record as Level 0.
- Level 1 = Lowest color in Level 1 = Record as Level 1.
- Level 2 = Lowest color in Level 2 = Record as Level 2, etc.

In Urine, a NicAlert™ result of **Level 3 (100-200ng/mL)** or higher indicates use of tobacco products: see Table 2a

In Saliva, a NicAlert™ result of **Level 1 (10-30ng/mL)** or higher indicates use of tobacco products: see Table 2b

The Accutest® NicAlert™ test strip may be read at any time following completion. When stored out of sunlight the strip can provide a stable record (>3years).

Expressing the Results as "Levels": Accutest® NicAlert™ was designed so that each Level corresponds to a concentration range for cotinine and/or 3-hydroxycotinine (see Table 1).

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

For purposes of assigning a semi-quantitative measure of tobacco exposure the Accutest® NicAlert™ level should be expressed in terms of "cotinine equivalents" as the test detects both cotinine and hydroxycotinine. Cotinine equivalents are the approximate concentration of cotinine and hydroxycotinine in the sample.

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

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

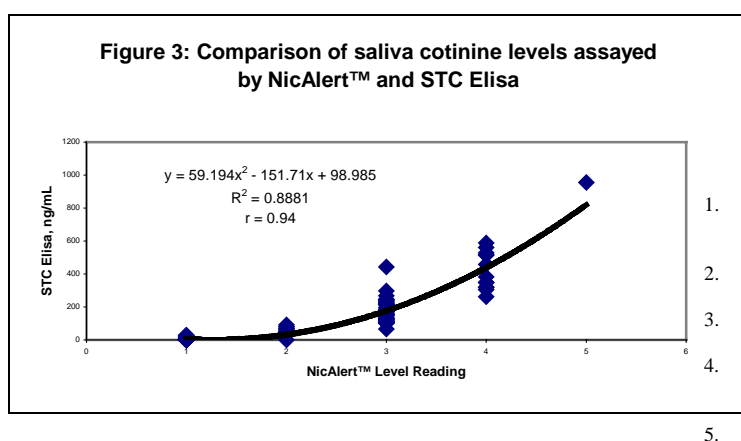
LIMITATIONS OF THE PROCEDURE

The Accutest® NicAlert™ is not intended for medical diagnostic or therapeutic purposes. Accutest® NicAlert™ is only for use with human saliva and urine. A positive result only indicates tobacco product exposure 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™ to the STC Elisa Assay

A panel of 150 saliva samples was field tested with the Accutest® NicAlert™ and the sample was sent to the laboratory and analyzed using the STC Cotinine Elisa. For detection of tobacco product exposure, a cut-off level of 10 ng/mL was used. The correlation between the Accutest® NicAlert™ and Elisa Assay was $r = 0.94$. See Figure 3 and Tables 2a and 2b.



Precision

Inter-strip variation

A negative saliva pool was spiked with cotinine to levels of 60, 300, and 2100 ng/mL. Each pool was tested 10 times with the Accutest® NicAlert™. All 10 replicates of the 60 ng/mL cotinine spike read Level 2 (30-100 ng/mL). All 10 replicates of the 300 ng/mL cotinine spike read Level 4 (200-500 ng/mL). All 10 replicates of the 2100 ng/mL cotinine spike read Level 6 (≥ 2000 ng/mL).

Day to Day variation

A pool of saliva from non-smokers, known to be negative for cotinine, was spiked with cotinine at levels of 60, 300 and 2100 ng/ml. Each pool was tested 10 times on 10 separate occasions over a 10-day period, with the Accutest® NicAlert™. On all ten occasions over

the 10 day period, the 60 ng/mL cotinine spike read in Level 2 (30-100 ng/mL). Likewise over the same period, on all 10 occasions the 300 ng/mL cotinine spike read in Level 4 (200-500 ng/mL) and the 2100 ng/mL cotinine spike read in Level 6 (≥ 2000 ng/mL).

Interferences

1. pH

Portions of a negative saliva pool (at pH 7.2) were adjusted with 1M citric acid to pH 4, 4.5, 5 and 6. Additionally 0.5 M sodium carbonate was used to adjust portions of the saliva to pH 8, 9, 9.5 and 10. Cotinine was then spiked into all salivas to a level of 150 ng/mL. All the spiked samples were tested immediately with Accutest® NicAlert™. The results are shown in Table 3.

Table 3: The effect of pH on NicAlert™

pH	NicAlert™ Reading	
	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 Accutest® NicAlert™ read as expected between pH 4 and 9.5. The normal pH range for saliva is 6.5 – 6.9, and saliva is reported to have a large buffer capacity⁽⁷⁾, i.e. it has a strong tendency to resist changes in pH.

2. Cross-reactants

Hydroxycotinine: Hydroxycotinine was spiked into a negative saliva pool to levels 0.125, 0.25, 0.5, 1, and 2 µg/mL. All of the solutions were tested with Accutest® NicAlert™. All levels of hydroxycotinine showed cross-reactivity ~25%.

Nicotine: Nicotine was spiked into a negative saliva pool to levels of 2, 10, and 20 µg/mL. All of the solutions were tested with Accutest® NicAlert™. The unspiked and 2 µg/mL spikes read Level 1. The 10 and 20 µg/mL spike read Level 2, indicating a maximum cross reactivity of <1%.

The following other pyridine derivatives were spiked into a negative saliva pool to a level of 50 µg/mL: niacinamide, nicotinic acid (niacin), nicotinic hydrazide, isonicotinic hydrazide, iproniazide phosphate, metyrapone, isonicotinic acid. All of the solutions were tested with NicAlert™ and read Level 0, which is a negative result.

3. Other Interfering Substances

Chlorpheniramine spiked into a negative saliva pool to a level of 200 µg/mL did not affect the assay; testing gave a reading of level 0, which is a negative result.

Glucose, ascorbic acid, albumin, and hemoglobin were spiked at 0 µg/mL (control) and either 500 µg/mL or 50 µg/mL into a saliva pool that was spiked to a cotinine level of 120 ng/mL (Level 3) and tested with Accutest® NicAlert™ (Table 4). All of these compounds read Level 3, which indicates that there was no interference from these substances that would affect the cotinine readings.

Table 4: Effects of Glucose, Albumin, Hemoglobin and Ascorbic Acid on NicAlert™

Substance Added	Spike Level; µg/ml	NicAlert™ Reading (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
Hemoglobin	0	3	3
	50	3	3

REFERENCES

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5. Murray R.P., et. al., "Error in Smoking Measures: Effects of Intervention on Relations of Cotinine and Carbon Monoxide to Self-Reported Smoking," Am. J. Public Health, 83: 1251-1257, 1993.
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7. Birkhed D., and Heintze U. Human Saliva: Clinical Chemistry and Microbiology, Tenovuo J., Odont D. (Eds.) CRC Press, Inc. Boca Raton, Florida, Vol. 1: pp. 52-55, 1989.

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