

NicCheck® I Test Strips

For Use in the Detection of Nicotine and/or its Metabolites in Urine

1.0 Catalog Number 500-001

2.0 Intended Use

NicCheck I Test Strips may be used to detect nicotine and/or its metabolites in urine as an aid in indicating the smoking status of the individual and in planning appropriate treatment. The test will also aid in the identification of a smoker as a low or high nicotine consumer.

The NicCheck I test is for professional use only. Individuals, such as physicians, nurses, pharmacists, medical technologists, and paramedical personnel may perform the test.

3.0 Summary and Explanation of the Test

3.1 Background

In recent years, the knowledge and awareness of health hazards associated with tobacco consumption (especially from smoking cigarettes) has increased. Surgeon Generals' Reports of the U.S. Public Health Service (PHS) have identified cigarette smoking as one of the most significant causes of death and disease in the U.S. This awareness has also increased all over the world. Smoking is cited as one of the major causes of cancer (1, 2); it is responsible for an estimated 30% of all cancer deaths including 87% of lung cancer, the leading cause of cancer mortality. It is also responsible for 21% of deaths from coronary heart disease, 18% of stroke deaths, and 82% of deaths from chronic obstructive pulmonary disease (3). Other forms of tobacco use, including pipe and cigar smoking and the use of smokeless tobacco are also associated with significantly elevated risks of disease and death (4, 5).

Self-reports of smoking behavior have been shown to be unreliable (6, 7, 8, 9). The availability of sensitive and reproducible analytical methods has led to the increased use of biochemical markers for the measurement of tobacco consumption (10). Measurement of carbon monoxide, carboxyhemoglobin or thiocyanate in the blood, or of nicotine/cotinine in plasma, saliva or urine have been used to validate self-reported smoking habits. However, none of these methods are satisfactory for routine use. Measurement of expired carbon monoxide or carboxyhemoglobin levels and thiocyanate levels may vary due to exposures unrelated to smoking, such as traffic emissions and diet (11). Nicotine is a tobacco-specific alkaloid with a half-life of 2 hours in blood. Its principal metabolite cotinine (12), has an average half-life of 20 hours in urine (13, 14), making it a reasonable candidate for use as a biochemical marker for tobacco consumption.

The NicCheck I test is a simple test that detects

nicotine and/or its metabolites in urine as a means to identify habitual consumers of tobacco. It can also differentiate nicotine consumers into "low" versus "high" categories. For the classification of smokers by the NicCheck I test as positive or negative based on comparison to GC urine cotinine values, individuals with cotinine values of 200 ng/mL and above were classified as smokers and those with cotinine values of less than 200 ng/mL were considered nonsmokers. The NicCheck I test is suited for routine use since it is technically simple to perform, and requires no instrumentation. Test results are available within 15 minutes.

There appears to be an increasing need to not only verify an individual's smoking status, but also to be able to know the level of nicotine consumption. The number of cigarettes smoked is not a true reflection of the nicotine consumed since the kinds of cigarettes smoked are different, the intensity with which cigarette smoke is inhaled can vary considerably from individual to individual, and the metabolism of nicotine in individuals can also vary. Thus, two individuals smoking the same number of cigarettes may have vastly different nicotine levels in their system. Determining the level of nicotine consumption, for example, can be important to aid individuals to quit their smoking habit. There is increasing evidence that a person with low nicotine dependence may require lower nicotine replacement therapy when compared to an individual who is more highly nicotine dependent. Additionally, monitoring the decrease in the color intensity on the NicCheck test strip may serve as an indicator of decreasing nicotine consumption during smoking cessation efforts and may provide the patient with positive reinforcement. The test may also be used to verify cessation. A test such as the NicCheck I test would provide a simple, inexpensive and rapid method for determining the smoking status of the individual and in identifying the smoker as a low or high nicotine consumer.

3.2 Chemical Principle of the Test Procedure

The NicCheck test strip has four chemicals spotted along the length of the strip at definite intervals. In testing for the presence of nicotine and its metabolites in urine, the NicCheck I test strip is placed (diethylthiobarbituric acid end first) into approximately 0.5 mL of urine contained in a suitable test tube. When urine diffuses up the test strip, the potassium thiocyanate mixes with chloramine-T on the strip, releasing cyanogen chloride. The cyanogen chloride then reacts with the nicotine (and/or its metabolites), if present, in the urine. Diethylthiobarbituric acid reacts with the resulting glutacetaldehyde to produce a pale pink to dark pink color along the length of

the test strip and in the liquid remaining at the bottom of the tube.

4.0 Reagents

4.1 Name and Quantity of the Product

NicCheck I test strips are packaged 50 to a canister. Each test strip is composed of the following:

- 7.5 cm x 0.5 cm absorbent paper impregnated with:
- Chloramine-T,
- Potassium thiocyanate,
- Citrate buffer, and
- Diethylthiobarbituric acid.

4.2 Warnings and Precautions

This product is intended "For in vitro Diagnostic Use."

There have been no reports of hazards associated with the appropriate use of NicCheck I test strips or with the use of NicCheck I Human Urine Positive and Negative Test Controls.

Body fluid specimens must be considered to be infectious and must be handled with appropriate precautions.

While performing the test, cyanogen chloride is produced in situ in a very small quantity as an intermediate. The concentration of this intermediate generated during the NicCheck I reaction is 2.3% of the reported inhalation limit in man (15) if exposed for 10 minutes. However, it should be mentioned that cyanogen chloride is toxic and an irritant. It may be harmful if swallowed or inhaled or absorbed through skin. Clothing, eye, and skin contact with the NicCheck I test strip must be avoided. If skin contact is made, the affected area must be flushed thoroughly with water. Vapors or mists or dusts must not be inhaled. The NicCheck I test should be run in a ventilated area. The test strip must not be ingested.

Chloramine-T and potassium thiocyanate are present on the strip in micromolar quantities. They may irritate the eyes or skin upon contact. They can cause allergic reaction if mishandled.

4.3 Handling Procedures

Each test strip should be carefully removed from the canister by only handling the strip at the arrow end. Handling other parts of the test strip must be avoided. Alternatively, a pair of clean forceps may be used to remove the strips from the canister. The test strip must be placed in the urine sample (approximately 0.5 mL) with the indicator arrow pointing downward in order for the urine sample to diffuse past the reagents in the proper sequence. The test sample container (a 13 x 100 mm glass tube is recommended) must be long enough to enclose the length of the entire test strip. The canister must be closed tightly after removal of the required number of strips.

4.4 Storage Instructions

NicCheck I test strips do not require refrigerated shipping. Upon receipt, the canister should be kept at 2–8° C. The test strips should be protected from unnecessary light and humidity to prevent light-induced and moisture-induced deterioration of the reagents on the test strip.

In general, when stored in the tightly closed canister at 2–8° C, the test strips can be used for at least two years from the date of manufacture. Since the test strips are susceptible to conditions of high humidity, the canister must be kept tightly closed after removal of the required number of strips.

4.5 Indications of Instability

While a pale brown color may be observed at the lower end of an unused test strip, this does not indicate a degradation of the test strip and does not interfere with the reaction. However, a bright yellow, purple, or dark brown color observed elsewhere on the test strip may indicate instability. Quality control checks using NicCheck I Human Urine Positive and Negative Test Controls (sold separately) should be performed whenever instability is suspected.

5.0 Specimen Collection and Preparation

NicCheck I test strips may be used with any freshly voided, stored (refrigerated or frozen) urine specimens or with urine collected under special conditions, such as first-morning specimens and post-prandial urine specimens. The urine specimen must be collected in a clean container. Preservatives must not be added to the urine specimen. If testing cannot be performed within 4 hours after collection of the urine, the specimen must be stored at 2–8° C. If stored at 2–8° C, testing must be performed within 48 hours. The specimens must be brought to room temperature prior to testing, and mixed thoroughly before use. The test may also be performed on specimens stored frozen at –20° C. The frozen specimens may not be frozen and thawed more than three times. Studies beyond three freeze-thaw cycles have not been conducted. If stored frozen, the specimens must be thawed and brought to room temperature prior to testing.

It should be noted that the NicCheck I test strips function appropriately in the pH range of 4.5–8.5. The NicCheck I test may react as false negative if the pH of the urine is outside of this range. Normal urine has a pH of 4.5–8.0 with an average pH of 6.0.

6.0 Procedure

6.1 Test Procedure

6.1.1

Obtain a urine specimen and transfer 0.5–1.0 mL of urine to a 13 x 100 mm test tube or equivalent. Cap tubes with 13 x 100 mm test tube caps (or cover) if needed. (See section 8.0, limitations of the test due to humidity.)

6.1.2

Remove a NicCheck I test strip from the canister, handling the strips only at the arrow end. Do not touch any other part of the test strip. Alternatively, a clean pair of forceps may be used to remove the test strip from the canister.

6.1.3

Place the test strip directly into the urine specimen with the arrow pointing downward into the specimen, and leave it there.

6.1.4

After introduction of the test strip into the specimen, observe results at 15 minutes. For differentiation into "low" versus "high" consumption of nicotine, comparison of the test results to the color chart provided must also be performed at 15 minutes.

6.2 Materials Provided

Fifty (50) NicCheck I test strips are provided with each kit. A color chart for the differentiation of smokers into "low" versus "high" consumers of nicotine is also provided with each kit.

6.3 Materials Required but Not Provided

6.3.1

13 x 100 mm test tubes (or equivalent).

6.3.2

Forceps, and caps for 13 x 100 mm test tubes (optional). Caps should be used if humidity is a concern or to improve sensitivity and consistency.

6.3.3

Test tube rack.

6.4 Quality Control Procedures

Good laboratory practice recommends periodic use of quality control procedures. It is recommended that the NicCheck I Human Urine Positive and Negative Test Controls (sold separately) be run at least once per day when clinical specimens are tested.

7.0 Interpretation of Results

The appearance of a pale pink to dark pink color on the strips is a positive reaction and indicates the presence of nicotine and/or its metabolites in the specimen. Occasionally, colors in the spectrum of orange to reddish pink may be observed. These are also to be considered as true positive reactions. Color development should occur along the length of the test strip. Occasionally, color will be pulled into the remaining urine sample turning a red or orange color. These reactions are to be considered positives and the samples should be retested. The intensity of color on the strip at the end of 15 minutes may be compared to those on the color chart, to differentiate between "low" versus "high" nicotine consumption.

If the color along the length of the test strip is less in intensity than the pink color on the color chart, the result is read as a "low" positive. If the

color on the test strip matches in intensity or is darker in intensity than the pink color on the color chart, the result is interpreted as a "high" positive. Absence of a color in the pink to red spectrum is considered a negative result.

Note: As the NicCheck I test reaction occurs both for positive and negative samples, it is normal for a bright yellow color to develop at the top end of the strip (near the arrows), this may also be accompanied by a purple/violet line and not meant to be used for interpreting results.

8.0 Limitations of the Procedure

If reactions with positive and negative test controls are not as expected, unused test strips should not be used, and the distributor and/or manufacturer should be notified.

Consumption of therapeutic levels (daily dose of 500 mg or greater) of niacin may result in a false-positive reaction by the NicCheck I test.

Reliable results and proper interpretation of results are dependent on performing the procedure as described in the package insert.

For the correct result to be obtained with the NicCheck I test, it is important to note the following conditions under which proper color development on the test strip may be impeded.

Delivering more than 1 mL of urine into the test tube for the NicCheck I test may result in improper color development due to inability of the sequential reaction to occur. Delivering less than 0.5 mL of urine will be insufficient to wet the full length of the test strip.

Using a beaker or urine collection container for performing the NicCheck® I test is unacceptable. Since the color development depends upon the sequential reaction of reagents along the entire length of the test strip, it is important to use a narrow test tube which is long enough to enclose the entire length of the strip.

For color development, the test strip must be placed with the arrow pointing downward into the tube.

Urine must be delivered directly to the bottom of the test tube in order to avoid sticking of the test strip to the inner wall of the tube. If the test strip sticks to the inner wall of the test tube, it does not allow for proper color development along the length of the test strip. The test must be repeated if this occurs.

Under conditions of high humidity, the test tube containing the NicCheck I test strip must be kept covered, preferably with 13 x 100 mm test tube caps. This option can also improve sensitivity and consistency.

The NicCheck I result must be read 15 minutes after introduction of the test strip into the urine. Reading the reaction after 25 minutes may result in a decrease in the color intensity on the NicCheck test strip.

9.0 Performance Characteristics

9.1 Clinical Data

9.1.1 Sensitivity and Specificity of the NicCheck I Test

The clinical evaluation of the NicCheck I test was performed at three clinical sites. A total of 249 smokers (categorized as "high" based on carbon monoxide (CO) in exhaled air of >20 ppm, or as "low" based on CO in exhaled air of 11–20 ppm) and 150 nonsmokers (CO in exhaled air of 0–10 ppm) were tested by the NicCheck I test and also by gas chromatography (GC) for cotinine and nicotine content in the urine.

The relative sensitivity and specificity of the NicCheck I test when compared to CO in exhaled air were 96.8% (with a 95% confidence interval of 93.8% to 98.6%), and 97.3% (with a 95% confidence interval of 93.3% to 99.3%) respectively (Table 1).

Table 1

NicCheck® I Results Compared to CO

When compared to urine cotinine values as determined by GC, the overall sensitivity and specificity of the NicCheck I test were 97.6% (with a 95% confidence interval of 94.8% to 99.1%) and 97.4% (with a 95% confidence interval of 93.4% to 99.3%), respectively (Table 2).

Table 2

	SITE			
	Arizona	Florida	Michigan	Overall
Relative Sensitivity	89/91 = 97.8%	80/80 = 100%	72/78 = 92.3%	241/249 = 96.8%
Relative Specificity	48/50 = 96%	49/50 = 98%	49/50 = 98%	146/150 = 97.3%

NicCheck® I results compared to cotinine by GC*

*For the classification of NicCheck I test results as positive or negative based on comparison to GC urine cotinine values, individuals with cotinine values of 200 ng/mL and above were classified as smokers, and those with cotinine values of less than 200 ng/mL were considered nonsmokers.

9.1.2 Differentiation of "Low" versus "High"

	SITE			
	Arizona	Florida	Michigan	Overall
Relative Sensitivity	90/91 = 98.9%	79/79 = 100%	72/77 = 93.5%	241/247 = 97.6%
Relative Specificity	49/50 = 98%	49/51 = 96.1%	50/51 = 98%	148/152 = 97.4%

Nicotine Consumption by the NicCheck I Test

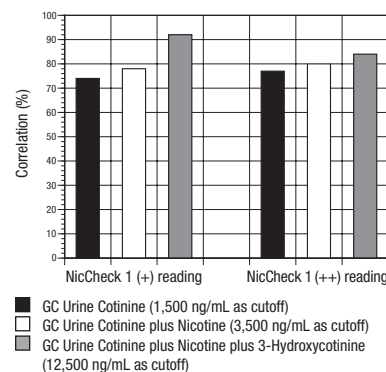
The data among those positive by NicCheck I and positive by GC (employing a cutoff value of 200 ng/mL of urine cotinine) were further analyzed to determine whether the NicCheck I test might provide a differentiation between levels of nicot-

tine consumption. It was determined that a "+" ("low") NicCheck I color reading correctly identified subject samples containing <1500 ng/mL of cotinine 73% of the time and the occurrence of a "+" ("high") NicCheck I color reading correctly identified subject samples containing ≥1500 ng/mL of cotinine 76% of the time. When the sum of nicotine and cotinine GC values in urine were taken into account, it was found that a "+" NicCheck I color reading correctly identified subject samples containing <3500 ng/mL of nicotine plus cotinine 78% of the time, and a "+" NicCheck I color reading correctly identified subject samples containing ≥3500 ng/mL of nicotine plus cotinine 80% of the time. Furthermore, when the urine GC values for 3-hydroxycotinine were included in the analyses, a "+" NicCheck I color reading correctly identified subject samples containing <12,500 ng/mL of nicotine plus 3-hydroxycotinine 90% of the time, and a "+" NicCheck I color reading correctly identified subject samples containing ≥12,500 ng/mL of nicotine plus 3-hydroxycotinine 92% of the time. Thus, when more of the metabolites are included in the analyses, there is a corresponding improvement in the overall correlation of a NicCheck I "low" or "high" result and the amount of nicotine metabolites in the urine. This has been depicted in Figure 1.

Figure 1

Comparison Between NicCheck I (+) and (++) Readings and Urine GC Values for Cotinine, Nicotine and 3-Hydroxycotinine

Since the NicCheck I test detects other nicotine metabolites in addition to cotinine and 3-hy-



droxycotinine, and also the fact that nicotine plus cotinine plus 3-hydroxycotinine constitute only 70–80% of the total nicotine metabolite pool in the urine, it can be concluded that the overall correlation of the NicCheck I test in classifying nicotine consumers as "low" versus "high", based on nicotine plus total metabolites present in the urine would approach 100% when the contribution of color provided in the NicCheck I reaction

by the remaining untested nicotine metabolites is taken into consideration.

Therefore, in view of the correlation between the intensity of color present on the NicCheck test strip and the total amount of nicotine and/or metabolites present in the urine which are detectable by GC, NicCheck I can be used as a practical means of differentiating low versus high consumption of nicotine.

9.2 Precision Testing

At each of the clinical trial sites, assay precision was assessed by examining the performance of the NicCheck I test in duplicate samples of 20 urine specimens provided by DynaGen on five separate occasions. They consisted of urines from 8 nonsmokers and 12 smokers. Testing was performed blind.

At each of the three sites, two trained individuals performed the NicCheck I test on a total of 40 samples (20 specimens tested in duplicate) once a day for a period of five days to establish assay precision. Thus, a total of 1200 assays were performed, of which 480 were expected to be negative since the samples were from nonsmokers, and 720 were expected to be positive with varying intensities such that some would be interpretable as "+" ("low") and some as "+" ("high").

All 480 negative samples were correctly classified as negative and all 720 positive samples were correctly identified as positive for an overall assay precision for a negative versus positive NicCheck I result of 100%.

For classification of the NicCheck I positive samples as "+" ("low") or "+" ("high"), out of the 720 positive samples, 698 were correctly classified for a precision of 96.9% in the identification of "low" versus "high" smokers.

9.3 Interfering Substances

9.3.1 Interference by various compounds added in vitro

One hundred thirty (130) compounds typically present in over-the-counter medications, as well as other drugs including drugs-of-abuse, were tested for their interference with the NicCheck I test. Normal urine from non-consumers of tobacco were spiked with 100,000 ng/mL or 500,000 ng/mL of the compound. The spiked specimens were tested with the NicCheck I test with and without any cotinine added. Pheniramine, brompheniramine and chlorpheniramine, which are antihistamines, were found to produce a false positive result in the NicCheck I test when tested at the 100,000 ng/mL concentration. At the recommended daily doses of antihistamines, the concentration of pheniramines that would be present in the urine would be significantly lower. When the NicCheck I test was performed on normal urine spiked with the expected level of pheniramines, there was no false positive result.

No other compound reacted with either a false positive or a false negative result in the NicCheck I test.

9.3.2 Interference by various medications

The effect of drug interference was evaluated separately among individuals who consumed nicotine in addition to concomitant medication for other medical conditions, as well as from nonsmokers who consumed various medications. For individuals who were non-consumers of nicotine, the NicCheck I test was run on their samples as is, and then with the addition of cotinine at the lower limit of detection of the NicCheck I assay.

Samples were tested from individuals consuming medications for diabetes, hypertension, cardiac conditions, epilepsy, depression, hypothyroidism, arthritis, and elevated cholesterol. Other medications consumed included sedatives, analgesics, antacids, antibiotics, decongestants, antihistamines, cold medicines, vitamins, estrogen supplements, and also drugs-of-abuse. The samples were classified into smokers versus nonsmokers based on the self-reported smoking status and by cotinine determination by enzyme immunoassay. There was one false positive result each among individuals who consumed medications for elevated cholesterol, arthritis hypertension, and antihistamines. Other individuals who consumed medications for the same conditions did not produce false positive results.

There were no false negative results by the NicCheck I test due to any of the medications consumed.

Additionally, data from the clinical trials were analyzed for the concomitant medications consumed. It should be noted that even with the consumption of medications for various medical conditions including diabetes, asthma, depression, heart condition, arthritis, acid reflux, hypothyroidism, elevated cholesterol, allergies and high blood pressure, as well as the consumption of various pain relievers, birth control pills, estrogen supplements and dietary supplements such as multivitamins, calcium and iron supplements, and prenatal vitamins, the specificity of the NicCheck I test was 97.3%.

9.3.3 Study for Interference due to Abnormal Urine Specimens

Urine samples with abnormal characteristics were also tested to determine whether these characteristics interfere with test results. Nonsmoker urine samples with abnormal levels of glucose, hemoglobin, protein, specific gravity, bilirubin, and uric acid crystals were tested with and without cotinine. Cloudy urine samples and urine samples with bacteria obtained from nonsmokers and smokers were also tested. None of the abnormal urine specimens, except for urine samples with bacteria, caused false positive or false negative results in the NicCheck I test. Three of the

eight bacterial samples from nicotine consumers produced false negative results. However, these three specimens had an abnormally high pH of 9. It should be noted that when tested at the lower limit of detection (3µg/mL cotinine), the optimal pH range for the NicCheck I test was determined to be 4.5–8.5.

10.0 Limit of Detection

The lower limit of detection of the NicCheck I test was determined in vitro by the addition of various amounts of nicotine and metabolites to aliquots of human urine obtained from nonconsumers of nicotine. Results were read by three trained individuals. The lowest concentrations at which a clearly discernable positive result was visible were as follows: 5 µg/mL for nicotine, 2.5 µg/mL for 3-hydroxycotinine, and 2.5 µg/mL for cotinine. Since the NicCheck I test is somewhat subjective, a conservative claim of 3 µg/mL has been established as the lower limit of detection for cotinine which would be readable by all observers. However, lower levels of detection may be observed by experienced readers. Limits of detection for other metabolites were also evaluated. Data are on file at Mossman Associates, Inc.

11.0 Expected Values

Approximately 46 million Americans smoke tobacco products. The NicCheck I test is designed to identify the habitual consumer of tobacco. The NicCheck I test was evaluated on an adult population who were further categorized as either smokers or non-smokers. An individual was classified as a nonsmoker based on having a carbon monoxide (CO) level in exhaled air equal to or less than 10 ppm using the CO Smokerlyzer meter. An individual was classified as a smoker based on a CO level greater than 10 ppm. Of the 249 smokers and 150 nonsmokers tested with the NicCheck I test, a positive predictive value of 98.4% and a negative predictive value of 94.8% were demonstrated.

Individuals were also classified as nonsmokers based on cotinine levels by gas chromatography (GC) equal to or less than 200 ng/mL; and as smokers based on cotinine levels greater than 200 ng/mL. Of the 247 smokers and 152 nonsmokers tested with the NicCheck I test, a positive predictive value of 98.4% and a negative predictive value of 96.1% were demonstrated.

12.0 References

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