Brief Report

Evaluation of NicCheck I Test Strips for the rapid detection of urine nicotine metabolites

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Abstract

Background: Tobacco use adversely affects surgical outcomes, prompting guidelines for preoperative smoking cessation. Accurate assessment of smoking status is critical, yet self-reporting is often unreliable. Biochemical verification via nicotine metabolites, particularly cotinine, offers objective evaluation. While mass spectrometry provides high sensitivity, point-of-care (POC) tests offer rapid, accessible alternatives. This study assesses the performance of NicCheck I, a CLIA-waived urine test, by comparing its results to liquid chromatography–tandem mass spectrometry (LC-MS/MS) for detecting tobacco exposure in surgical patients.

Methods: Twenty-four frozen urine samples (12 cotinine-negative, 12 cotinine-positive) were analyzed using NicCheck I test strips and LC-MS/MS. Sample integrity, and pH were assessed using Siemens Multistix 10 SG reagent strips.

Results: NicCheck I correctly identified all negative samples but detected nicotine metabolites in only 9 of 12 LC-MS/MS-confirmed positive samples. False negatives were associated with metabolite concentrations below the NicCheck I detection threshold (200 ng/mL) or urine pH outside the optimal range (4.5 – 8.5) consistent with manufacturer's package insert. LC-MS/MS demonstrated superior sensitivity.

Conclusion: NicCheck I test strips provide a practical and same-day rapid screening tool for nicotine exposure in clinical settings prior to surgery, but the test is less sensitive than LC-MS/MS. NicCheck I test strip performance may be affected by abnormal urine pH.

Introduction

Tobacco use remains a major public health challenge, and a leading preventable cause of disease, death, and disability both in the United States and globally. In the United States alone over 16 million people are living with a smoking-related disease resulting in more than 480,000 deaths each year [1].

In surgical care settings, smoking is strongly associated with adverse perioperative outcomes, including poor wound healing, higher risk of infection, cardiac and pulmonary complications, longer hospital stays, and increased overall healthcare costs [2,3,4]. Recognizing these risks, the World Health Organization recommends smoking cessation at least four weeks prior to surgery, noting that each additional week of abstinence reduces postoperative complications by approximately 19% [5]. While the optimal timeline for quitting smoking before surgery remains debated, the American Society of Anesthesiologists Task Force on Smoking Cessation emphasizes that patients should abstain from smoking for as long as possible both before and after surgery [6].

Accurate assessment of a patient's smoking status is essential for perioperative risk stratification and surgical planning. Self-reported smoking history, while commonly used, is often unreliable due to underreporting or misrepresentation, particularly in settings where smoking may influence surgical eligibility or outcomes [7, 8]. Biochemical verification through detection of nicotine and/or its metabolites in blood or urine offers a more objective and reliable alternative.

Nicotine, the primary alkaloid in tobacco, has a very short half-life (1-4 hours). In humans, approximately 70-80% of nicotine is rapidly metabolized by the cytochrome P450 2A6 (CYP2A6) in the liver to its primary metabolite, cotinine, which has a longer half-life (16-20 hours). Approximately 10-15% of the cotinine is excreted in the urine. Remaining cotinine is further metabolized to six primary metabolites including trans-3'-hydroxycotinine (3-OH-cotinine) which has a half-life of ~15 hours [9]. Cotinine and 3-OH-cotinine are considered reliable biomarkers for tobacco use due to their specificity, extended half-lives, and elevated urinary concentrations [10]. Importantly, cotinine concentrations in urine, saliva or serum are reflective of the amount of nicotine exposure [11].

Tobacco smoke exposure can be detected using various biochemical techniques, including gas chromatography (GC) and liquid chromatography (LC) coupled with mass spectrometry (MS), as well as immunological assays. Among these, MS-based methods offer high sensitivity and specificity for identifying nicotine and its metabolites. However, MS requires technical expertise, has long turnaround times, is expensive and is not widely available in many clinical laboratories. In contrast, point-of-care (POC) testing devices have emerged as practical alternatives for rapid, same-day screening for tobacco consumption. These Food and Drug Administration (FDA)-cleared and Clinical Laboratory Improvement Amendments (CLIA) -waived tests utilize colorimetric detection to provide qualitative or semi-quantitative results for nicotine metabolites in urine [8].

Given the importance of timely and accurate nicotine screening, particularly in plastic and reconstructive surgery where smoking cessation is often mandated, POC testing represents a valuable tool in perioperative care [12].The efficiency of NicCheck I (Mossman Associates, Inc, Milford, MA) test strips has previously been assessed against breath carbon monoxide (CO) and has since achieved a CLIA waived status [13]. This study aims to evaluate the analytical performance of NicCheck I test by comparing results for urine nicotine and its metabolites with those obtained through liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods

Specimens

Frozen urine samples (n=24) were obtained from ARUP laboratories (Salt Lake City, UT). These included negative (n=12) samples for cotinine by Enzyme Multiplied Immunoassay technique and positive (n=12) samples for nicotine and metabolites confirmed by quantitative LC-MS/MS.

Testing

Frozen urine samples were thawed at room temperature and centrifuged (2000 rpm for 5 minutes). NicCheck I positive/negative cotinine controls and urine samples were tested using the NicCheck I test strips following manufacturer's instructions in the package insert. The NicCheck I test strip detects nicotine in urine through a chemical reaction. Urine activates reagents on the strip, producing cyanogen chloride, which reacts with nicotine and/or its metabolites. This forms glutaconaldehyde, which then reacts with diethylthiobarbituric acid on the strip, resulting in a pink color that indicates the presence of nicotine and/or metabolites. Color developed on the test strip should be observed after 15 minutes and the intensity of color development can be compared to a color card provided by the manufacturer. Color intensity in the range of 1-6 or 7-12 as indicated on the color card are classified as low consumer and high consumer of nicotine respectively.

Sample pH and integrity were simultaneously assessed on Clinitek status analyzer using Siemens Multistix 10 SG reagent strips (Tarrytown, NY).

Results

NicCheck I test strips incubated with cotinine negative urine samples (n=12) did not develop any color after 15 minutes, indicating absence of nicotine and/or metabolites. NicCheck I strips incubated with LC-MS/MS confirmed positive urine samples developed a pink color in 9 out of the 12 samples indicating the presence of nicotine and/or metabolites (Table 1). According to the manufacturer's instruction for use, NicCheck I test strips can detect cotinine >200 ng/mL (as determined by Gas chromatography) with a positive predictive value of 98.4%.

Table 1: Association between qualitative color development on NicCheck I test strips, urine pH, and LC-MS/MS–measured cotinine and nicotine metabolite levels in urine samples (n=12) that tested positive for nicotine and its metabolites by LC-MS/MS.

Sample	Color development (range)	Urine pH (Clinitek)	Cotinine (ng/mL) (LC-MS/MS)	Nicotine and metabolites (ng/mL) (LC-MS/MS)
1	**Absent**	**6**	**32**	**<102**
2	Present (1)	5	330	988
3	Present (1)	5.5	587	1889
4	**Absent**	**6**	**17**	**<83**
5	Present (6-7)	6	1299	>4705
6	Present (1-2)	5.5	595	1560
7	Present (5-6)	5.5	1007	>4464
8	Present (7-8)	7	803	>3584
9	**Uneven coloration**	**≥9**	**1063**	**>3156**
10	Present (6-7)	5.5	1292	>3759
11	Present (2-3)	6.5	771	>2820
12	Present (1)	5	378	>2412

Samples 1 and 4 showed no color development and had low or near-below-detection analyte levels (<200 ng/mL), whereas increasing color intensity was associated with progressively higher cotinine and nicotine metabolite concentrations. Uneven coloration was observed in one urine sample (sample 9; pH ≥9) even with high analyte levels.

Discussion

With increasing awareness of the detrimental effects of smoking on postoperative outcomes, there is a growing need for rapid and simple POC testing to detect nicotine and its metabolites on the day of and immediately prior to surgery. Such testing has the potential to enhance perioperative decision-making, improve patient compliance, and ultimately optimize surgical outcomes. However, the availability of CLIA-waived tests for nicotine detection remains limited. To date, no studies have directly compared the performance of NicCheck I test strips with the LC-MS/MS.

In this study, we evaluated the analytical performance of NicCheck I test strips relative to LC-MS/MS. Our findings suggest that NicCheck I strips are adequate for initial screening of urine samples for nicotine and its metabolites. However, their sensitivity is inferior to LC-MS/MS, particularly for samples with lower concentrations of total nicotine metabolites which may result in false-negative results. This limitation underscores the importance of confirmatory testing in clinically ambiguous cases.

Additionally, urine pH was identified as a potential confounding factor, consistent with the manufacturer's package insert. One sample with a pH ≥9 yielded an inconclusive NicCheck I result despite having high concentrations of nicotine and its metabolites (>3156 ng/mL) as confirmed by LC-MS/MS. This observation suggests that urine pH should be routinely assessed when interpreting test results obtained using NicCheck I test strips.

Importantly, NicCheck I test strip does not distinguish between different tobacco sources or passive smoke exposure. For patients utilizing nicotine replacement therapy (NRT), confirmation of tobacco abstinence requires testing for anabasine, a tobacco-specific minor alkaloid absent in NRT products. LC-MS/MS remains the preferred method for anabasine detection and should be employed when precise differentiation is clinically necessary. Additionally, NicCheck I test strips detect all nicotine metabolites - including 3-hydroxycotinine which is the most abundant metabolite of nicotine (~40%) in urine and may persist for up to two weeks after smoking cessation [14]. This might cause some individuals to test positive even after recent abstention from smoking. Nonetheless, given that smoking cessation is typically recommended for at least 4 weeks if not more prior to surgery, the test retains clinical utility in identifying recent tobacco exposure [5,12].

One limitation of this evaluation was the small sample size. However, the number we evaluated was sufficient to delineate the differences in cutoff concentration between NicCheck I and LC-MS/MS methods, as well as define the potential for interference from urines with pH outside manufacturer recommended ranges. In conclusion, while NicCheck I test strips offer a reliable and rapid screening tool for nicotine exposure in the perioperative setting, their limitations necessitate cautious interpretation. When clinical decisions rely

on accurate smoking status - particularly in patients using NRT or presenting borderline results, LC-MS/MS should be utilized to ensure diagnostic precision.

Author Contributions

Seema Khattri Bhandari (Conceptualization- equal, investigation-lead, formal analysis-equal, data curation-equal, writing- original draft- lead). James H Nichols (Conceptualization-equal, formal analysis- equal, data curation-equal, Writing- review and editing, supervision-lead).

Conflicts of interest

The authors declare that they have no conflicts of interest related to this work.

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Data availability

The data supporting the findings of this article can be made available upon request.

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