Evaluation of a Urine Screen for Acetaminophen

Daniel M. Ingram, MD, MSPH^a, George M. Bosse MD^a, Edward P. Womack, Jr BS MT (ASCP)^b, Saeed A. Jortani, PhD^b

^aDepartment of Emergency Medicine, University of Louisville School of Medicine, Louisville, KY

^bPathology and Laboratory Medicine, University of Louisville School of Medicine, Louisville, KY

ABSTRACT

Background: Acetaminophen (APAP) is a leading cause of fatal overdose. This study examined the performance characteristics of the Biosite Triage TOX Drug Screen qualitative APAP urine test (urine screen) in a clinical setting.

Methods: Paired urine and serum waste samples (collected within 2 hours of one another) were quantitatively analyzed for APAP concentration and compared to the urine screen results.

Results: A total of 191 paired samples met inclusion criteria. The urine screen did not report a negative result for the 21 samples with a serum APAP concentration of $\geq 10\mu\text{g/mL}$ and had a sensitivity of 100% (95% confidence interval [CI], 85–100%). The urine screen had a sensitivity of 96% (95% CI, 91–99%) and a specificity of 90% (95% CI, 82–95%) for detecting urine APAP concentrations $> 5\mu\text{g/mL}$ by gas chromatograph mass spectroscopy (GCMS) methods, and a sensitivity of 81% (95% CI, 73–87%) and a specificity of 95% (95% CI, 85–98%) by high-pressure liquid chromatography (HPLC) methods.

Conclusions: The urine screen detected urine APAP concentrations with good accuracy and may be an effective screen to rule out acute APAP overdose in some circumstances. Its clinical utility is discussed.

INTRODUCTION

Acetaminophen (APAP), i.e., N-acetyl-*p*-aminophenol, is a common ingredient in many over-the-counter and prescription medications. It has an excellent safety profile if taken as directed. However, overdose is common, in part due to both availability and widespread public ignorance of APAP's potential toxicity. It is by far the most common cause of acute liver failure in the United States [1].

Therapeutic doses of APAP are predominantly metabolized by conjugation to inactive metabolites. A small percentage is metabolized to the hepatotoxic compound N-acetyl-*p*-benzoquinoneimine (NAPQI), which is detoxified by endogenous glutathione. Following APAP overdose, excess NAPQI is formed, which overwhelms both glutathione stores and production [2]. The antidote, N-acetylcysteine (NAC), is most effective if initially administered within 8–12 hours after overdose [3]. Unfortunately, during this

time period the signs and symptoms of acetaminophen toxicity may be vague, nonspecific or absent. By the time clinical signs of hepatotoxicity are evident, usually within a few days, liver damage may be irreversible and fatal.

Overdose patients may not be forthcoming about the nature and quantity of their ingestions owing to altered mental status, the desire to overdose successfully, ignorance that APAP may be dangerous, or lack of knowledge that APAP is present in their medications. Clinicians should have a high index of suspicion for APAP overdose. The National Academy of Clinical Biochemistry Laboratory Medicine Practice Guidelines recommend routine screening for acetaminophen in patients presenting to the emergency department (ED) with intentional drug ingestion [3].

The diagnosis and treatment of acute APAP overdose traditionally relies on the serum APAP concentration drawn at 4 to 24 hours after ingestion and the utilization of the Rumack–Matthew Nomogram [4]. However, some facilities lack the capability to

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Corresponding Author: Saeed A. Jortani, PhD, 511 South Floyd Street (Room 204), Department of Pathology and Laboratory Medicine, University of Louisville School of Medicine Louisville, KY 40202. Phone: 505-852-8835. Fax: 502-852-7674. Email: sjortani@louisville.edu

perform quantitative serum analysis for APAP in-house and must send it out for analysis, though the prevalence of these facilities is unknown. They may have difficulty in obtaining the result in a timely manner in order to make appropriate patient disposition decisions. Physicians in these settings may needlessly transfer patients who did not overdose or not order a serum APAP concentration in patients who have overdosed. As a diagnosis of APAP overdose is not always elicited by the medical history and physical examination, there are advantages to routinely screening for APAP overdose even in larger centers [3].

However, the yield of routine acetaminophen screening is low, with one large study showing that in patients presenting with altered mental status or suicidal ingestion the percentage of patients with serum APAP concentrations of greater than 1 µg/mL was only 9.6%. While only 0.3% had potentially toxic serum APAP concentrations without a suggestive history, the authors considered this sufficient reason to screen all suspected overdose patients [5].

This study examined the performance of one such test, the new APAP assay available on the Biosite Triage TOX Drug Screen (urine screen), one of the various test devices that may be run on the Biosite Triage MeterPlus. The study was designed to evaluate the performance of the urine screen in a clinical setting, specifically to determine how well it detected urine concentrations of acetaminophen ≥ 5 µg/mL and how well these results correlated with serum APAP concentrations. Two important issues are considered: the question of what is a reasonable gold standard for measuring APAP in urine, and how to use a urine screen for a condition in which the serum concentration is the basis of the standard of care.

METHODS

The urine screen is a competitive fluorescence immunoassay used for the qualitative determination of the parent compounds and likely for major metabolites in urine specimens. After the addition of a urine sample to the test device, the urine passes through a filter, moves by capillary action into a reaction chamber, and reacts with fluorescent antibody conjugates or mixes with fluorescent drug conjugates to form a reaction mixture. After an incubation period, this flows through the device detection lane. The presence of drug or drug metabolites in the urine specimen prevents binding of the fluorescent conjugates to the solid phase on the detection zone. Excess urine washes unbound fluorescent conjugates from the detection lane into a waste reservoir. The device reads the fluorescence bound to the detection lane and reports a positive or negative result. The test is internally set to read positive when it detects concentrations of at least 5 µg/mL. Test results are available in approximately 15 minutes.

Study Design and Setting

Paired urine and serum samples in adult patients (collected within 2 hours of one another) were collected over a 9-month

period. Times between collection of urine and serum samples were recorded. Patients whose urine and serum collection times differed by more than 2 hours were excluded. Unfortunately, the time from possible ingestion to the time when the samples were obtained is not known, though this is often the case in clinical practice as well. Hospital and University Institutional Review Board approval as a waste-sample study was obtained, so gender and age data were not available.

Methods of Measurement

All serum samples were analyzed by our routine quantitative serum APAP immunoassay on a COBAS Integra instrument (Roche Diagnostics, Indianapolis, IN) with a lower limit of detection of 0.7 µg/mL. Both the method and the instrument are FDA approved for measuring APAP in serum, and the method has a coefficient of variation of $\leq 7.5\%$ for the concentration range of 9.9 to 97.4 µg/mL. Urine samples were analyzed by the urine screen. Urine APAP concentrations were also determined by high-pressure liquid chromatography (HPLC) done at our facility and gas chromatography mass spectroscopy (GC/MS) methods performed on blinded samples at Biosite, Inc. The HPLC and GC/MS methods each have a lower limit of detection of 1 µg/mL.

HPLC Methods

For HPLC analysis, 1 mL of urine was extracted using ammonium hydroxide. The extracted sample was reconstituted and run on the HPLC system composed of a C-18 reverse-phase column by Waters Corporation (Milford, MA) with UV detection at 242 nm. Semiquantitative concentrations of APAP in the urine were then determined using a single-point calibration at 5 µg/mL of APAP. In this HPLC method, the parent compound as well as metabolites and possibly other similar substances may coelute at the retention time corresponding to acetaminophen.

PRIMARY DATA ANALYSIS

Statistical analysis was performed using STATA 8.0 statistical software (Stata Corporation, College Station, TX). Performance characteristics of the urine screen were computed for their ability to detect urine APAP, using both HPLC and GC/MS results as possible gold standards. Sensitivity, specificity, and positive and negative likelihood ratios were computed. However, negative and positive predictive values were not computed, as these depend on having a clear denominator, which this waste sample study does not have.

Performance characteristics were also computed for the urine screen's ability to predict the serum APAP concentration. Serum concentration was divided into 5 clinically relevant categories: below our limit of detection (<0.7 µg/mL), 0.7–10 µg/mL, 10–30 µg/mL, 30–140 µg/mL, and >140 µg/mL. Performance characteristics were computed using each of these cutoff points to allow clinicians the freedom to determine their own level of

clinical relevance. However, these cutoffs also relate specifically to acute ingestions and are easier to interpret if the time from ingestion is known, which in this study it is not.

RESULTS

A total of 286 paired urine and serum samples were collected. Of these, 57 were excluded due to collection time differences of greater than 2 hours. Thirty-eight samples were excluded due to either being duplicate samples from the same patient or insufficient quantity for analysis. A total of 191 paired serum and urine samples met our inclusion criteria.

To assess the ability of the urine screen to detect APAP in the urine, the results of the urine screen were compared to HPLC and GC/MS, with results shown in *Tables 1, 2* and *3*. *Table 1* shows both results together so that the degree of agreement or disagreement may be noted, and the other two show the same results separately for clarity. There were 158 of 191 samples (82.7%) for which there was agreement between the HPLC and GC/MS results, yielding a kappa of 0.63.

The performance characteristics of the urine screen for detecting urine APAP at a concentration of 5 µg/mL were computed and are shown in *Table 4*. Compared to HPLC, the urine screen had a sensitivity of 81% (95% confidence interval [CI], 73–87%), a specificity of 95% (95% CI, 85–98%), a positive likelihood ratio

of 15 (95% CI, 5–45) and a negative likelihood ratio of 0.20 (95% CI, 0.14–0.28). Compared to GC/MS, the urine screen had a sensitivity of 96% (95% CI, 91–99%), a specificity of 90% (95% CI, 82–95%), a positive likelihood ratio of 10 (95% CI, 5–19) and a negative likelihood ratio of 0.04 (95% CI, 0.016–0.11).

As shown in *Table 2*, 29 of 191 patients (13.6%) had different results by HPLC and the urine screen. Of these, 26 patients had a positive urine HPLC but a negative urine screen. Four had detectable serum APAP concentrations, with values of 1.9, 3.4, 6.5, and 8.0 µg/mL. They had urine concentrations of APAP by GCMS ranging from 0 to 5.8 µg/mL. None received NAC treatment.

As shown in *Table 3*, 12 of 191 patients (6.3%) had different results by GC/MS and the urine screen. Of these, 4 patients had a positive GC/MS result but a negative urine screen, and 3 of these were included in the 26 patients that also had a positive HPLC but a negative urine screen. By GCMS, their urine concentrations were 5.2, 5.8, 7.0, and 10.7 µg/ml. Three of the 4 had undetectable serum APAP concentrations and 1 had a serum APAP concentration of 8 µg/mL. None receive NAC treatment.

Urine screen results were compared to the serum APAP concentration at various cut-offs and are shown in *Table 5*, with performance characteristics computed from this table and shown in *Table 6*. If one considers a subtherapeutic serum APAP concentration (<10 µg/mL) to be negative, then the urine screen had a sensitivity of 100% (95% CI, 85–100%). The sensitivity of the urine screen to predict APAP serum concentrations above our limit of detection (0.7 µg/mL) was 87% (95% CI, 76–93%). No patients with a negative urine screen had a serum APAP >10 µg/mL.

Table 1: Urine screen qualitative results vs. HPLC and GC/MS urine APAP concentrations

HPLC	Urine Screen			Total
	GC/MS	(–)	(+)	
(–)	(–)	52	1	53
(–)	(+)	1	2	3
(+)	(–)	23	7	30
(+)	(+)	3	102	105
Total		79	112	191

For HPLC and GC/MS columns: (–) is <5 µg/mL, (+) is ≥5 µg/mL.

Table 2: Urine screen qualitative results vs. HPLC urine APAP concentration

HPLC	Urine Screen		Total
	(–)	(+)	
(–)	53	3	56
(+)	26	109	135
Total	79	112	191

For HPLC and GC/MS columns: (–) is <5 µg/mL, (+) is ≥5 µg/mL.

Table 3: Urine screen qualitative results vs. GC/MS urine APAP concentration

GC/MS	Urine Screen		Total
	(–)	(+)	
(–)	75	8	83
(+)	4	104	108
Total	79	112	191

For HPLC and GC/MS columns: (–) is >5 µg/mL, (+) is ≥5 µg/mL.

Table 4: Performance characteristics of urine screen for the two reference assays

Urine Screen	HPLC	GC/MS
Sensitivity	81% (73–87%)	96% (91–99%)
Specificity	95% (85–98%)	90% (82–95%)
+LR	15 (5–45)	10 (5–19)
–LR	0.20 (0.14–0.28)	0.04 (0.016–0.11)

LR = likelihood ratio

Parentheses enclose 95% confidence intervals.

Table 5: Urine screen qualitative result vs. serum APAP concentration

Urine Screen	Serum APAP concentration $\mu\text{g/mL}$					Total
	<0.7 *	0.7–9.9	10–30	30.1–139	≥ 140	
(–)	71	8	0	0	0	79
(+)	60	31	13	6	2	112
Total	131	39	13	6	2	191

* Limit of detection.

Table 6: Performance characteristics for the urine screen when predicting serum APAP concentration at various cutoffs

Urine Screen	How well the urine screen predicts a serum APAP level above this cutoff value, in $\mu\text{g/mL}$			
	>0.7?*	>10?	>30?	>140?
Sensitivity	87% (76–93%)	100% (85–100%)	100% (67–100%)	100% (34–100%)
Specificity	54% (46–62%)	46% (39–53%)	45% (36–50%)	43% (35–49%)
+LR	1.9 (1.5–2.3)	1.9 (1.6–2.1)	1.8 (1.6–2.0)	1.7 (1.5–1.9)
–LR	0.25 (0.13–0.48)	0 (**)	0 (**)	0 (**)

* Limit of detection
** Confidence intervals unstable
LR = likelihood ratio
Parentheses enclose 95% confidence intervals.

However, this makes the zero negative likelihood ratios shown in Table 6 unreliable due to the cell containing a zero, and the computation of confidence intervals around these results is not straightforward.

DISCUSSION

In suspected acute acetaminophen overdose cases, use of serum concentration is the widely accepted standard in therapeutic decision making and patient management. Routine acetaminophen screening is recommended in all suspected drug overdose cases [3]. Recently, a qualitative urine APAP test was introduced as part of the Triage TOX Drug Screen by Biosite Incorporated intended for use at the point-of-care.

This study was designed to answer two questions: 1) Does the urine screen accurately detect urine APAP concentrations $\geq 5 \mu\text{g/mL}$, and 2) does the urine screen perform as a clinically reliable negative rule-out screen for acute APAP overdose?

Regarding the first question, the urine screen results more closely matched the GC/MS results than the HPLC results, possibly indicating that the urine screen's antibodies are more specific for the parent compound than metabolites. Both sensitivity (96% [95% CI, 91–99%]) and specificity (90% [95% CI, 82–95%]) were high by GC/MS. Those 4 patients with positive GC/MS results but

negative urine screens had urine APAP concentrations by GC/MS that were very close to the cutoff of $5 \mu\text{g/mL}$. Thus, the answer to the first question is that the basic chemistry of the test functioned properly and did detect urine APAP.

The second question: that of the clinical utility of a urine screen for acetaminophen, is significantly more complex than the first. The data available in this study showed that no one with a negative urine screen had a serum APAP concentration consistent with a treatable level of APAP overdose, at least by the Rumack-Matthew Nomogram and assuming acute ingestion less than 19 hours prior. However, no cases in this sample with a negative urine screen went on to exhibit clinical signs of APAP toxicity. Thus, in this sample, it functioned as an accurate rule-out screen for acetaminophen. There were 8 cases with negative urine screens and detectable acetaminophen concentrations less than $10 \mu\text{g/mL}$. These individuals may have required antidotal therapy in certain settings and in acute ingestions ≥ 19 hours earlier. The need for an inexpensive and rapid rule-out screen for acute ingestions is evident when looking at the low percentage of patients with higher serum APAP concentrations, which we define as $>30 \mu\text{g/mL}$. By the Rumack-Matthew Nomogram, a serum APAP concentration $>30 \mu\text{g/mL}$ may be clinically relevant at 4–13 hours after acute ingestion. In our study, only 8 of 196 (4.2%) of our patients had concentrations this high.

LIMITATIONS

The total number of known or treated APAP overdoses in the study was low, reflecting what is commonly found in clinical practice. Urine concentrations inherently lag behind serum concentrations, making it theoretically possible that very recent overdoses might be missed if urine is obtained almost immediately after drug ingestion, although we did not find this problem in our facility. In addition, testing of urine from 14 healthy volunteers 30 minutes postingestion of a 1000-mg dose of acetaminophen resulted in positive APAP results in all cases with the urine screen [6]. Further, a patient's voiding frequency and hydration status may alter results of any urine drug screen.

That an APAP urine screen can be positive when the serum concentration has dropped below detectable amounts could raise the suspicion of recent APAP overdose in a patient with liver failure of unknown etiology or in a polysubstance overdose patient who is unable to give a history. As there is evidence that NAC may be effective 24 hours or longer after acute, toxic ingestions of APAP [7], a positive urine screen might prompt either unnecessary or lifesaving treatment in a patient with a negative serum concentration and a suspicious presentation.

Unfortunately, with this waste sample design and acetaminophen being ordered as a part of large, comprehensive toxicological panels in patients with no history of overdose or unknown time from ingestion, we have insufficient data on time from possible ingestion until our serum and urine samples were obtained. Thus, this study can only comment on these issues, in theory.

As the urine screen is part of a panel of routine drugs of abuse, wider screening for APAP becomes possible, especially in settings in which serum testing is not readily available. Therefore, presence of a positive urine APAP result might prompt questioning about ingestions that otherwise might have been missed, or prompt checking a serum APAP concentration when one might not have been obtained.

The question of what to do with chronic APAP ingestions is a complex one, even when considering serum APAP concentrations. The urine screen for APAP could confirm the presence of APAP and possibly prompt liver function tests and checking a serum APAP concentration.

However, despite these limitations, we found that the urine screen was an effective negative rule-out screen. Attempts at providing a straightforward economic analysis of the test are complicated by a number of factors, among them being that each Triage TOX Drug Screen test device includes assays for multiple

drugs, as well as the difficulties in computing the actual laboratory cost of the alternatives, such as a quantitative instrument that performs many individual analyses. However, as it can be challenging for clinicians to obtain the laboratory results in adequate time to impact disposition decisions, the urine screen, with rapid results at the bedside, can potentially be a valuable tool to enhance patient flow in the ED. While the urine screen had a very high sensitivity for detecting serum APAP concentrations, its specificity was lower (approximately 50% depending on what cutoff is used), with a positive result requiring a follow-up test to determine the actual serum concentration.

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