Comprehensive Review

Clinical Benefits of Direct-to-Definitive Testing for Monitoring Compliance in Pain Management

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Background: The technical advantages of direct-to-definitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) urine testing for monitoring patient compliance in pain management are well known. However, the design and implementation of LC-MS/MS methods are more controversial, including factors such as determining appropriate cutoffs, specimen processing (e.g., specimen hydrolysis), reporting of qualitative and/or quantitative results, and test menu.

Objectives: The objective of the research was to compare the clinical performance of our previous urine pain toxicology panel, a combination of immunoassay (IA) screens and LC-MS/MS, to our current pain toxicology panel, which features direct-to-definitive LC-MS/MS for 34 drugs and metabolites.

Study Design: Six months of results from our previous pain toxicology panel were compared to 5.5 months of results from our current pain toxicology panel, enabling us to make conclusions regarding clinical performance.

Setting: The research took place at Brigham and Women's Hospital in Boston, MA.

Methods: The percentage of false positive IA results was evaluated for our previous pain toxicology panel. The positivity rates for each drug and/or metabolite were calculated for both the previous and current panels, including rates of detection of both prescribed and illicit drugs. The turnaround time (TAT), direct and send-out costs associated with each approach, as well as projected cost savings were also determined.

Results: False positive rates with IA ranged from 0% to 29%; the highest false positive rate was seen for 6-acetylmorphine (6-AM). The elimination of IA, addition of metabolites, and/or lowering of cutoffs increased the detection rate of 6-AM, benzoylecgonine (cocaine metabolite), fentanyl, morphine, and oxycodone. The ability to differentiate compliance from simulated compliance improved after eliminating specimen hydrolysis. The TAT improved significantly and projected yearly cost savings with the current panel was $95,003 (USD). In our opinion, qualitative results appeared sufficient to assess compliance in the majority of cases.

Limitations: Our study was performed in a single academic center in a specific geographic region; therefore, our results may not be generalizable to other types of centers or regions.

Conclusion: Direct-to-definitive LC-MS/MS testing has several clinical benefits, including reduction of false positive results, improved assessment of patient compliance, decreased TAT, and increased detection of drug use and abuse. Cost savings were also realized using this approach.

Key words: Direct-to-definitive, LC-MS/MS, immunoassay, sensitivity, cost, pain management, turnaround time, patient compliance

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Every day, approximately 150 Americans die from a drug overdose (1,2). Furthermore, it is estimated that 29% of patients prescribed opioids misuse them, and 8-12% develop a chemical dependence (1,2). In 2015, more than 33,000 people died from an opioid overdose in the United States (1,2). The effects of the opioid epidemic are presenting unique challenges to the US medical community. Providers must effectively manage pain while simultaneously assessing compliance and preventing prescription misuse. Urine drug testing, in combination with other tools, is commonly used by providers for this purpose.

There are 2 major methodologies for urine drug testing: immunoassay (IA) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (3,4). IA is widely utilized because it is relatively easy to implement and can provide rapid turnaround time (TAT) (4-6). However, IA has notable limitations, including cross-reactivity with drugs outside the targeted drug class, suboptimal analytical sensitivity and specificity, inability to detect specific drugs, and the need for a relatively large sample volume (100-200 µL) (5). IA also has a higher cost per sample compared to LC-MS/MS (4,6-13). For these reasons, recent pain management guidelines and literature suggest bypassing IA and performing more sensitive and specific direct-to-definitive testing by LC-MS/MS (3,5,9-10,12,14-15).

Reported advantages of LC-MS/MS include lower detection cutoffs for analytes to allow for longer drug detection, ability to report parent drugs and related metabolites to allow for more accurate identification of drug(s), smaller sample volumes, and lower reagent costs (9,12,14,16-18). However, transitioning to direct-to-definitive clinical testing may be challenging. LC-MS/MS platforms are relatively expensive to purchase and maintain, and technologists must be adequately trained to validate, operate, maintain, and troubleshoot equipment. Furthermore, the testing volume may not justify analyzing specimens on-demand, which can limit the utility of LC-MS/MS platforms in TAT-sensitive areas such as the emergency department. Despite these challenges, our group has previously shown that in-sourcing LC-MS/MS is achievable and can be cost-effective at a tertiary academic medical center (10,19).

The technical advantages of LC-MS/MS have been well studied (9,12,14,16-21). However, the clinical utility of LC-MS/MS is less clear: What are appropriate cutoffs for drug detection? Should specimen hydrolysis of conjugated analytes (e.g., morphine glucuronides) be performed prior to analysis? Should qualitative and/or quantitative results be reported? Which parent drugs and metabolites should be included in a test menu? In this paper, we report the clinical utility, cost effectiveness, and operational benefits of direct-to-definitive LC-MS/MS testing for monitoring compliance in pain management and discuss design of LC-MS/MS methods.

Methods

Previous Pain Toxicology Panel

Before August 2017, the clinical laboratory at Brigham and Women's Hospital (Boston, MA, USA) utilized a combination of (1) direct-to-definitive LC-MS/MS testing for benzodiazepines and opiates/opioids (drugs/drug classes with light gray shading in Table 1) using our previous first-generation method, (2) IA with reflex to definitive testing for positive results (drugs/drugs classes with * in Table 1), and (3) IA with reflex to definitive testing for positive results if requested by the provider (drugs with ** in Table 1). Methadone and 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidne (EDDP) were only sent for definitive LC-MS/MS testing if the IA results for these drugs were discrepant. The 6-acetylmorphine (6-AM; heroin metabolite) IA screen was only performed if morphine levels were greater than 2000 ng/mL by LC-MS/MS assay.

Qualitative IA screens were performed on a Beckman AU480 analyzer (Beckman Coulter Inc., Brea, CA) using a homogenous enzyme immunoassay (HEIA) (Immunalysis Corporation, Pomona, CA) for fentanyl and tramadol; a Diagnostic Reagents Inc. (DRI) enzyme immunoassay (Microgenics, now Thermo Scientific, Fremont, CA) for amphetamines, cocaine metabolite, and methadone; an enzyme immunoassay (EIA) (LinZhi International, Sunnyvale, CA) for 6-AM and buprenorphine, and a cloned enzyme donor immunoassay (CEDIA) (Thermo Scientific, Fremont, CA) for EDDP. Qualitative cutoff concentrations for each IA are listed in Table 1. With the exception of tramadol, positive IA results were automatically sent to a reference laboratory to be confirmed by LC-MS/MS or GC-MS testing.

Our previous first-generation laboratory-developed LC-MS/MS method was utilized for benzodiazepines and opiates/opioids. Samples were prepared by adding an internal deuterated standard to the following drugs or metabolites: 7-aminoclonazepam, alpha-hydroxy-alprazolam, lorazepam, nordiazepam, oxazepam, temazepam, codeine, hydrocodone, hydromorphone, morphine, oxycodone, and oxymorphone. Urine samples were diluted and subjected to enzymatic

Table 1. *Comparison of previous and current urine pain toxicology panels.*

Drug Class	Drug	Cutoff Previous Panel (IA* or MS) (ng/mL)	Cutoff Current Panel (MS) (ng/mL)	Current Panel Qual/Quant Results
Amphetamines*	Amphetamine	1000**	25	Qual
	MDA		25	Qual
	MDMA		25	Qual
	Methamphetamine		25	Qual
Benzodiazepines	7-aminoclonazepam	50	25	Quant
	Alpha-hydroxy-alprazolam	50	25	Quant
	Clonazepam	N/A	5	Qual
	Diazepam	N/A	5	Qual
	Lorazepam	50	25	Quant
	Nordiazepam	50	25	Quant
	Oxazepam	50	25	Quant
	Temazepam	50	25	Quant
Buprenorphine*	Buprenorphine	5**	5	Quant
	Norbuprenorphine		5	Quant
	Buprenorphine-glucuronide		5	Quant
	Norbuprenorphine-glucuronide		5	Quant
	Naloxone		100	Quant
Cocaine Metabolite*	Benzoylecgonine	300**	5	Qual
Fentanyl*	Fentanyl	1**	2	Qual
	Norfentanyl	N/A	2	Qual
Methadone*	Methadone	300**	5	Qual
	Methadone Metabolite (EDDP)	100**	5	Qual
Opiates/Opioids	6-acetylmorphine (heroin metabolite)	10**	5	Qual
	Codeine	100	25	Quant
	Hydrocodone	100	25	Quant
	Hydromorphone	100	25	Quant
	Morphine	100	25	Quant
	Morphine-3-beta-glucuronide	N/A	25	Quant
	Morphine-6-beta-glucuronide	N/A	25	Quant
	Noroxycodone	N/A	25	Quant
	Oxycodone	100	25	Quant
	Oxymorphone	100	25	Quant
Tramadol**	O-desmethyltramadol	N/A	25	Qual
	Tramadol	200***	5	Qual

Qual = qualitative; Quant = quantitative; IA = immunoassay; MS = Mass Spectrometry; MDA = 3,4-methylenedioxyamphetamine; MDMA = 3,4-methylenedioxymethamphetamine; EDDP = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine
Light gray shading = direct to LC-MS/MS for previous panel; * = MS not performed unless IA screen positive for previous panel; ** = stated cutoff is for IA; *** = MS not performed, regardless of result, unless requested by the provider for previous panel; N/A and dark gray shading = not detected by IA screen and added to current MS panel

hydrolysis to remove glucuronide and sulfate groups. Chromatographic separation was achieved on an ACQUITY UPLC I-Class (Waters, Milford, MA) using a Kinetex C18 analytical column (Phenomenex Inc., Torrance, CA). Mass spectrometric analysis was performed on a tandem triple quadrupole Xevo TQS (Waters, Milford, MA) preceded by heated electrospray ionization (HESI). LC-MS/MS cutoff concentrations are shown in Table 1. Quantitative results were reported for all benzodiazepines and opiates/opioids.

Current Pain Toxicology Panel

In the current second-generation method, 34 drugs and related metabolites are analyzed by LC-MS/MS (Table 1). Urine samples are diluted with water and spiked with deuterated internal standards without an enzymatic hydrolysis. Samples are injected by an autosampler, and analytes are then separated using a CORTECS C18 column (Waters, Milford, MA) on an ACQUITY UPLC I-Class system (Waters, Milford, MA). Eluted analytes are ionized by HESI and fragmented using collision-induced dissociation to quantify specific parent-daughter ion transitions for each analyte and internal standard on a triple-quadrupole Xevo TQS system (Waters, Milford, MA). Compared to the previous first-generation LC-MS/MS assay, 7 additional drugs and metabolites were added: clonazepam, diazepam, norfentanyl, morphine-3-beta glucuronide, morphine-6-beta glucuronide, noroxycodone, and *O*-desmethyltramadol; these were either not measured in the previous panel or would not have triggered a positive IA screen (Table 1). Cutoff concentrations for the current panel using LC-MS/MS are shown in Table 1. Table 1 also indicates whether quantitative or qualitative results are reported.

Data Collection and Analysis

Results of the previous pain toxicology panel were obtained from the laboratory information system (LIS) for a period of 6 months (February 2017-July 2017). Likewise, results of the current pain toxicology panel were obtained from the LIS for an interval of approximately 5 months (August 14, 2017-December 2017). This study was approved by the Partners Human Resource Committee.

The IA false-positive rate for drugs in the previous pain toxicology panel was calculated by dividing the number of negative LC-MS/MS results by the total number of specimens sent for LC-MS/MS testing (i.e., those that screened positive by IA). Specimens that could not be reported by LC-MS/MS due to an interference were excluded from the calculation. Methadone and EDDP were not included in the analysis because only discrepant IA results were sent for LC-MS/MS. Similarly, tramadol was not included because few specimens were sent out for confirmation. Potential causes of false-positive results were determined by reviewing the package insert and the patients' medications.

The percentage of positive results by LC-MS/MS was compared between the previous and current pain toxicology panels for 6-AM, alpha-hydroxy-alprazolam, amphetamines, benzoylecgonine (cocaine metabolite), buprenorphine (and/or metabolites), clonazepam (and/or metabolites), diazepam (and/or metabolites including nordiazepam, oxazepam, and temazepam), fentanyl (and/or metabolites), hydromorphone, lorazepam, and oxymorphone. Since the current panel includes morphine glucuronides and noroxycodone, we assessed the ability of the previous and current panels to detect compliance with morphine and oxycodone by comparing: (1) the percentage of patients positive by LC-MS/MS for both morphine and hydromorphone in the previous panel to the percentage of patients positive for morphine-3-beta-glucuronide and/or morphine-6-beta-glucuronide in the current panel and (2) the percentage of patients positive by LC-MS/MS for both oxycodone and oxymorphone in the previous panel to the percentage of patients positive for noroxycodone in the current panel.

In addition, we examined whether patients who had been deemed compliant with the previous panel by morphine positivity only (i.e., no hydromorphone detected) and/or oxycodone only (i.e., no oxymorphone detected) were still deemed compliant with our current panel. Patients were selected for this comparison if more than one sample was positive for morphine only or oxycodone only during the study period. The number and percentage of these patients who also had detectable metabolites in the current pain toxicology panel (i.e., morphine-3-beta-glucuronide or morphine-6-beta-glucuronide for morphine or noroxycodone for oxycodone) were calculated.

The ability to identify illicit drug combinations was assessed for the previous and current panels by calculating positivity rates for the following: 6-AM and fentanyl, cocaine and fentanyl, cocaine and 6-AM, and morphine and fentanyl.

Finally, the average TATs from specimen collection to result for the previous and current pain toxicology panels were determined. The percentage of results available within 7 days was also calculated. The two-

sample t test was used to analyze the results with a *P* value of less than 0.05 being considered significant.

Cost Analysis

Direct costs and send-out costs associated with the previous and current pain toxicology panels were calculated in USD. Direct costs included the instrument lease(s), reagents and consumables, labor, and service contract. Indirect costs were not included. Projected savings attributed to the current pain toxicology panel were also calculated.

Results

During the study period, a total of 1,674 specimens were analyzed using the previous pain toxicology panel (IA plus first-generation LC-MS/MS), and a total of 1,253 specimens were analyzed using the current pain toxicology panel (second-generation LC-MS/MS, no IA).

As shown in Table 1, the major differences between the 2 panels are that, with the current panel, (1) all testing is performed by LC-MS/MS only, (2) additional parent drugs and related metabolites are included, (3) the positivity cutoff is lower for many of the drugs/metabolites measured, (4) specimens are not hydrolyzed prior to analysis, and (5) a combination of quantitative and qualitative results are reported.

Immunoassay False-Positive Rate

The false-positive rate for IA using the previous pain toxicology panel ranged from 0% to as high as 29% (Table 2). The false-positive rate was lowest for benzoylecgonine at 0% (0/82) followed by buprenorphine at 7% (28/415), fentanyl at 16% (30/194), and amphetamines at 17% (12/72). The false-positive rate was highest for 6-AM at 29% (2/7), although the number of specimens sent for LC-MS/MS testing was low compared to the other drugs/drug classes in our analysis. Potential causes for the false-positive results are listed in Table 2.

Differences in Positivity Rates

The positivity rates varied between the previous and current pain toxicology panels. The rates increased for 6-AM, benzoylecgonine, and fentanyl (and metabolites), from 0.3% to 1.0%, 5% to 13%, and 10% to 13%, respectively (Table 3). For amphetamines, buprenorphine, and clonazepam (and metabolites), the positivity rates remained the same: around 4%, 24%, and 13%, respectively. The current panel does not detect glucuronides for alpha-hydroxy-alprazolam, diazepam metabolites, hydromorphone, lorazepam, or oxymorphone, which may contribute to a lower positivity rate (4% to 0.4% for alpha-hydroxy-alprazolam, 17% to 7% for diazepam [and metabolites], 10% to 8% for hydromorphone, 12% to 0.6% for lorazepam, and 3% to 0.6% for oxymorphone) (Table 3).

In addition, morphine and oxycodone metabolites were added during the creation of the current panel to help distinguish actual from simulated compliance with prescribed medications. Specifically, morphine-3-beta-glucuronide and morphine-6-beta-glucuronide were added for morphine, and noroxycodone for oxycodone, compliance assessment. The previous panel included only hydromorphone and oxymorphone as evidence of compliance with morphine and oxycodone, respectively.

Table 2. *False positive rate for immunoassay screens with previous pain toxicology panel.*

Drug/Drug class	Immunoassay Cutoff (ng/mL)	Immunoassay False Positive Rate % (Absolute)	Potential Interferences/Causes of False Positive Results
6-acetylmorphine (Heroin Metabolite)*	10	29 (2/7)	Triprolidine
Amphetamines	1000	17 (12/72)	Trazodone
Benzoylecgonine (Cocaine Metabolite)	300	0 (0/82)	n/a
Buprenorphine	5	7 (28/415)	Heroin, Levorphanol, EMDP
Fentanyl	2	16 (30/194)	Labetalol, Trazodone, Methamphetamine

*6-acetylmorphine was only measured when morphine levels were > 2000 ng/mL by LC-MS/MS
EMDP = 2-ethyl-5-methyl-3,3-diphenyl-1-pyrroline

Table 3. *Positivity rate for previous and current pain toxicology panels.*

Drug/Drug Class	Previous Pain Toxicology Panel % Positive (Absolute)	Current Pain Toxicology Panel % Positive (Absolute)	Change; Possible Explanation
6-acetylmorphine (Heroin Metabolite)	0.3 (5/1674)	1 (11/1253)	Increase; increase in number of specimens analyzed
Alpha-Hydroxy-Alprazolam	4 (62/1674)	0.4 (5/1253)	Decrease; current assay does not detect glucuronides
Amphetamines	4 (59/1674)	4 (47/1253)	Same; most specimens have high concentrations of amphetamines detectable by either panel
Benzoylecgonine (Cocaine Metabolite)	5 (81/1674)	13 (169/1253)	Increase; increased analytical sensitivity
Buprenorphine	23 (387/1674)	24 (297/1253)	Same; no changes to analytical parameters
Clonazepam and/or Metabolites	13 (211/1674)	13 (164/1253)	Same; slight increase in analytical sensitivity
Diazepam and/or Metabolites	17 (288/1674)	7 (85/1253)	Decrease; current assay does not detect glucuronides
Fentanyl and/or Metabolites	10 (164/1674)	13 (165/1253)	Increase; increased detection of norfentanyl
Hydromorphone	10 (170/1674)	8 (165/1253)	Decrease; current assay does not detect glucuronides
Lorazepam	12 (204/1674)	0.6 (7/1253)	Decrease; current assay does not detect glucuronides
Oxymorphone	3 (46/1674)	0.6 (8/1253)	Decrease; current assay does not detect glucuronides

Since both of those metabolites can also be found as separate formulations, compliance with morphine and oxycodone can only be inferred when both the primary drug and metabolite are detected in the same sample. On the other hand, noroxycodone and morphine glucuronides would only be expected to be present in the urine as a product of primary drug metabolism. Using the previous panel, the positivity rate for morphine and the minor metabolite, hydromorphone, was 10% (Fig. 1). The addition of the glucuronides increased the positivity rate to 14%. Similarly, the positivity rate for oxycodone and the minor metabolite, oxymorphone, was 31% with the previous panel; the addition of noroxycodone increased the positivity rate to 36% (Fig. 1).

Eight patients were positive for morphine only, without hydromorphone, in more than one specimen tested by the previous panel. With the current panel, glucuronide metabolites were detected in 7 of the 8 patients (88%). The last patient did not have detectable metabolites with either panel. Likewise, 5 patients were positive for oxycodone only, without oxymorphone, in more than one specimen from the previous panel. With the current panel, noroxycodone was detected in 3 of the 5 patients (60%). Two patients did not have oxymorphone or noroxycodone detected in either panel. Finally, there was an increase in illicit drug detection with the current pain toxicology panel. Detection of combined 6-AM and fentanyl increased from 0.2% to 1.1%, detection of combined cocaine and fentanyl increased from 0.8% to 4.1%, detection of combined cocaine and 6-AM increased from 0.2% to 0.6%, and detection of combined morphine and fentanyl increased from 3.9% to 4.6% (Fig. 2).

Turnaround Time and Cost Analysis

The average TAT for the previous panel was 7.2 ± 2.4 days. The TAT was significantly shorter at 5.2 ± 2.2 days ($P < 0.001$) for the current panel. The percentage of specimens resulted within 7 days increased from 42% with the previous panel to 72% with the current panel. Direct costs and send-out costs were both higher for the previous pain toxicology panel, totaling $336,996 per year (Table 4). The elimination of send-out costs, the equipment lease, and reagents for the IA analyzer

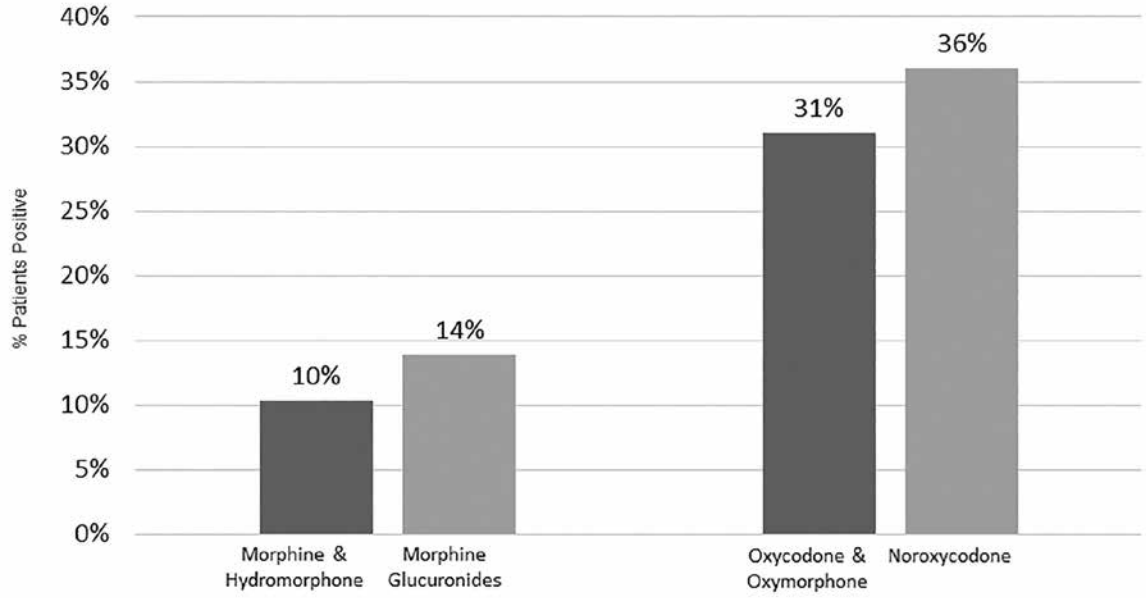

Fig. 1. *Positivity rates for drugs and their metabolites with the current and previous pain toxicology panels. The dark gray bars represent the positivity rate with the previous panel and the light gray bars represent the positivity rate with the current panel. Percentage of patients positive for both morphine and hydromorphone are depicted for the previous panel and percentage of patients positive for morphine-3-beta-glucuronide and/or morphine-6-beta-glucuronide are depicted for the current panel. Percentage of patients positive for both oxycodone and oxymorphone are depicted for the previous panel and percentage of patients positive for noroxycodone are depicted for the current panel.*

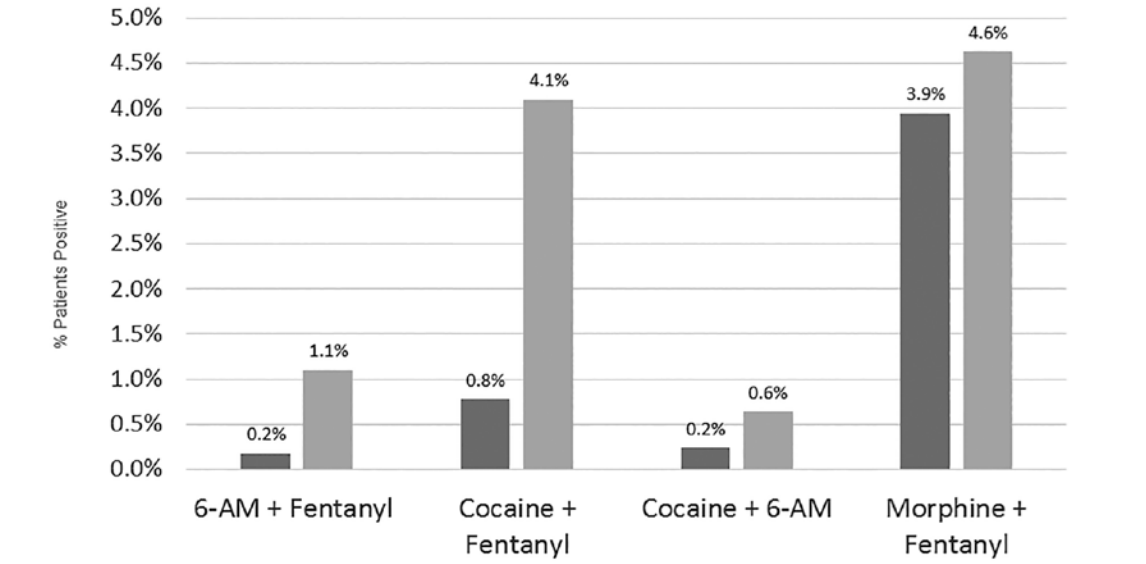

Fig. 2. *Positivity rate for illicit drug combinations with the current and previous pain toxicology panels. The dark gray bars correspond to the positivity rate with the previous panel and the light gray bars correspond to positivity rate for the current panel. Positivity rates for the following combinations of illicit drugs are shown: 6-AM and fentanyl; cocaine and fentanyl; cocaine and 6-AM; and morphine and fentanyl.*

Table 4. *Cost savings associated with current pain toxicology panel.*

Pain Toxicology Panel	Annual Direct Cost	Annual Send-out Cost	Total
Previous (Immunoassay & LC-MS/MS)	$283,496	$53,500	$336,996
Current (LC-MS/MS only)	$241,993	$0	$241,993
Projected Savings	$41,503	$53,500	$95,003

resulted in $95,003 projected yearly cost savings for the current pain toxicology panel (Table 4).

Discussion

Our 5-month experience with direct-to-definitive LC-MS/MS testing for 34 drugs and metabolites demonstrated several clinical benefits: improved assessment of patient compliance, increased detection of illicit drug use, decreased TAT, and significant direct cost savings.

Consistent with published literature, false-positive rates for IA in our previous pain toxicology panel were as high as 29% (6,10,13). Bypassing IA reduces the possibility of clinicians acting on false positive results. Furthermore, the lower limit of quantitation (LLOQ) of the LC-MS/MS method allows a higher detection rate for many drugs and/or metabolites, including 6-AM, benzoylecgonine, fentanyl, morphine, and oxycodone. Importantly, as the United States faces the challenges of the opioid crisis, this method offers an improved detection rate for illicit drug combinations such as 6-AM/fentanyl and cocaine/fentanyl. The lower cutoffs have also increased the window of detection for drugs and/or metabolites in urine. For example, the positivity rate for benzoylecgonine increased from 5% to 13% in our patient population, allowing detection of more remote cocaine use. Further, one patient who was given intravenous morphine during a procedure was positive for morphine metabolites for up to 2 weeks. Lowering the LLOQ will likely warrant re-investigation of drug and/or metabolite detection windows in urine (i.e., how long can we detect drug use in urine).

With the previous assay, 6-AM was analyzed only in specimens with morphine > 2000 ng/mL. Currently, 6-AM is measured on all specimens for which the pain toxicology panel is ordered. Interestingly, some patients have detectable 6-AM with either undetectable morphine or morphine < 2000 ng/mL, suggesting that 6-AM should be measured in all patients regardless of the morphine results.

Positivity rates for amphetamines, buprenorphine, and clonazepam (and related metabolites) were similar for the current and previous panels. Despite bypassing the IA screen and lowering the cutoff to 25 ng/mL for amphetamines, approximately 4% of our patients were positive for this class of drugs. This is likely due to amphetamine concentrations typically being high enough to trigger a positive result even at the 1000 ng/mL IA cutoff, regardless of whether it is being abused or prescribed. Similarly, lowering the cutoff from 50 to 25 ng/mL and adding clonazepam did not change the positivity rate. For buprenorphine, neither the test menu nor the cutoff changed, explaining the similar positivity rates for the previous and current panels.

Unlike our previous panel, no specimen hydrolysis is performed prior to analysis with the current pain toxicology panel. Our data support the notion that measuring both the parent drugs and their metabolites improves compliance monitoring. This is because detection of the metabolites in addition to the parent drug rules out simulated compliance (i.e., dropping the drug directly in the urine). Using the current panel, we discovered that of the 11 patients with questionable morphine or oxycodone results, 3 patients were simulating compliance with either morphine or oxycodone, while 8 patients were compliant with their prescribed medications. In our patient population, patients most frequently simulate compliance with buprenorphine. The measurement of naloxone and the glucuronide metabolites has also improved the ability of the laboratory and providers to differentiate compliance from simulated compliance.

The TAT for the panel improved after in-sourcing all the testing, which is particularly important for the initial weekly monitoring of patients prescribed buprenorphine. With the current panel, approximately three quarters of providers have their patient results before the next appointment. We plan to add another day of testing to further improve the TAT.

The lack of specimen hydrolysis in the second-generation LC-MS/MS assay used in the current panel may contribute to lower positivity rates for some drugs that are primarily excreted as glucuronides in urine. For example, the detection rate decreased for alpha-hydroxyalprazolam, diazepam metabolites (i.e., nordiazepam, oxazepam, temazepam), hydromorphone, lorazepam, and oxymorphone. Future iterations of the panel will include oxazepam-glucuronide, hydromorphone-

glucuronide, lorazepam-glucuronide, and alprazolam. The lower detection rate for oxymorphone was not concerning in our patient population, as the concentrations of oxymorphone in patients taking oxymorphone were detectable, and noroxycodone allowed assessment of oxycodone metabolism and compliance.

The current LC-MS/MS panel provides a combination of quantitative and qualitative results depending on the drug and/or metabolite. In our opinion, if all appropriate metabolites are included in the panel, qualitative results should be sufficient for most cases. Qualitative results also discourage providers from utilizing urine concentrations to predict time of last dose, which might be inappropriate and misleading (20). However, there may be some cases in which quantitative results are necessary to assess the quantity of parent drug ingested and/or to normalize the results for serum creatinine concentrations. Our current panel provides quantitative analysis of benzodiazepines, buprenorphine, and opiates/opioids. This is to ensure equivalency with the previous panel, and because providers felt that a quantitative result offered stronger evidence of non-compliance when presented to a patient with aberrant behavior.

Conclusion

We found that a close collaboration with our clinicians was important in order to determine the appropriate test menu to monitor compliance in their patient population. The test menu depended on medications prescribed, type of clinics (e.g., pain management, addiction, cancer-related pain), and illicit drug use patterns. For example, we included diazepam in our test menu to help providers determine whether the presence of nordiazepam, oxazepam, and/or temazepam was secondary to diazepam ingestion or another benzodiazepine that metabolizes to these compounds. Targeted LC-MS/MS methods such as the one described in this study are beneficial and widely utilized, but their test menu is more restricted and must be thoughtfully designed. It is our practice to re-evaluate the composition of the panel yearly.

Our findings suggest that in urine pain management testing, cutoffs should be determined by limits of quantitation of the assay, specimens should not be hydrolyzed prior to analysis (assuming glucuronides are included in the test menu), and qualitative results are sufficient in most cases. Further studies are necessary to determine the optimal detection windows for drugs and/or metabolites in urine using direct-to-definitive LC-MS/MS testing. A study focused on the clinical utility of quantitative versus qualitative results – including effect on patient counseling, validity of parent/metabolite ratios, and utility of creatinine normalization – would be helpful. Finally, each laboratory needs to assess the utility of LC-MS/MS and appropriate method for their patient population.

Direct-to-definitive LC-MS/MS testing has several clinical benefits including elimination of false-positive results, improved assessment of compliance, decreased TAT, and increased detection of drug use and abuse.

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