The Journal of the International Federation of Clinical Chemistry and Laboratory Medicine

eJIFCC

The Laboratory's Role in Opioid Pain Medication Monitoring

Geza S. Bodor
Veterans Administration, Eastern Colorado Health Care System (VA ECHCS), Denver

Corresponding Author:

University of Colorado Denver
Department of Pathology UCD – School of Medicine
Mail Stop 8104
Aurora, CO 80045--508
Tel.: +1-303-399-8020 x2625
e-mail: geza.bodor@va.gov

Key words

Opiates, opioids, pain medication monitoring, immunoassay, morphine, codeine, semi-synthetic opiates, LCMSMS, urine drug screen

Abstract

Opioid analgesics are the most potent pain medications therefore they are often used for the treatment of chronic malignant and non-malignant pain. Their strong addictive potential requires close monitoring of patients on opioid therapy for possible non-compliance with prescriptions, for drug diversion, and for proof of avoidance of non-prescribed or illicit opioids. Monitoring can be performed by urine drug screens or qualitative or quantitative drug confirmation assays. Natural, semi-synthetic and synthetic opioids have dissimilar chemical structures and they undergo extensive metabolism. Phase one metabolic reactions of opioids can produce other opioids with similar structures to other, non-prescribed medications. Only detailed and concurrent analysis of parent drugs and metabolites can provide accurate clinical information regarding patient compliance. Traditional immunoassays, often used for urine drug screening, react with only a small number of opioids or only with a single medication and they exhibit variable cross reactivity with their phase two metabolites. Additionally the limit of detection of these immunoassays may not be sufficient for medical purposes, therefore clinical interpretation of immunoassay test results can be challenging. Recently liquid chromatography, mass spectrometry (LCMSMS) based assays have been adapted by many clinical laboratories. These LCMSMS tests can provide information about the presence of several opioids and their metabolites in a single sample at clinically meaningful detection limits, allowing accurate assessment of patient compliance. This review article will investigate in details the various opioids, their metabolism and the challenges the testing laboratories and ordering clinicians face.

Chronic pain affects a significant portion of the US population and seeking treatment for chronic pain accounts for a large number of office visits annually (1). When drug therapy is necessary for pain control, the WHO 3-step analgesic treatment protocol is followed. This protocol requires the assessment of severity of pain, followed by a decision regarding which step of the treatment protocol should be applicable. Step 1, for mild pain, should be treated with non-narcotic analgesics, such as acetylsalicylic acid, acetaminophen or other NSAIDs. Steps 2 and 3 of pain severity require the coadministration of narcotic and non-narcotic analgesics, or even administration of more than one narcotic pain medications and adjuvant therapy (2). Narcotic analgesics that exert their action through the opioid receptors are commonly called opiates or opioids, and they are extensively used to control step 2 or step 3 severity pains. While they are excellent analgesics, they can also have significant side effects. Constipation,

nausea, vomiting, sedation, respiratory depression and coma are the common physiologic side effects and signs of possible opioid overdose. Respiratory depression and coma can lead to death, while sedation, combined with vomiting can cause aspiration pneumonia or the patient can suffocate while unconscious. Tolerance, physical dependence and addiction (psychological dependence) are other side effects of opioids that are exhibited differently by different individuals. Tolerance is the gradual development of resistance of the body to the analgesic effect of opioids, requiring increasingly higher doses of the drug for the same amount of pain relief. Tolerance can also be lost if the opioid drug is discontinued, even if for a few days, or for some opioids, even after missing just one or two doses. Resuming treatment at the previously tolerated dose after a hiatus could, therefore, lead to opioid overdose, with occasional fatal outcome. Physical dependence is caused by the adaptation of tissues to the effects of drug and can lead to withdrawal syndromes following sudden discontinuation of opioids or after treatment with antagonists. Addiction is characterized by drug seeking behavior, criminal activity to obtain the drug, dysfunctional opioid use manifesting in loss of control over the use of the drug, and/or the concurrent use of more than one narcotics, including illicit ones. Only a small number of patients will develop addiction who are taking opioid drugs for cancer pain, but the risk is higher for non-cancer related chronic pain patients and for persons with previous history of substance abuse (3).

To navigate the treacherous waters between the Scylla and Charybdis of insufficient pain control and addiction or severe to fatal drug overdose, laboratory testing is often ordered for the measurement opioid drug concentration in hope of guiding prescription practices. The United States and some other countries also recommend opioid drug testing to assess patient's compliance with prescription and to detect the presence of non-prescribed opioids as a sign of possible addiction. Some programs, such as the one by the US Veterans Administration, specifically require the assessment of the presence of prescribed drugs while expects non-prescribed drugs to be absent from patients on opioid pain medications. Violation of either of these requirements could result in discharge from the chronic pain management program because documented absence of the prescribed drug is interpreted as "diversion" or the illegal sale of prescription medication, and the presence of non-prescribed opiates is considered to be proof of illicit drug use and thus addiction.

Finally, opioid drug testing can also be performed for forensic purposes when a patient on opioid therapy dies unexpectedly and the suspicion of opioid overdose rises. Although forensic drug testing is outside of most clinical laboratories' scope of work, a clinical laboratory still may test antemortem samples from a patient who is transported to a hospital. If the patient were to expire in spite of the medical efforts the clinical laboratory's results can be subpoenaed in court where the analytic methods, the results and their interpretations could fall under legal challenge.

This paper is intended to briefly review the present state of opioid drug testing and to investigate if our current laboratory practices are satisfactory to answer the challenges of the analytic, medical and legal expectations.

Brief review of the chemistry and metabolism of opioid pain medications

Opiates and Opioids

Opioids are chemicals that exhibit morphine-like action in the body by binding to opioid receptors that are found in the central nervous system and in the gastrointestinal tract. There are three major types of opioid receptors, mu, beta and kappa, and each one has multiple subtypes. The action of a particular opioid depends on what type of receptor it can bind to, and if the opioid is an agonist or an antagonist of that receptor. None of the opioids have exclusive specificity for a single receptor, therefore their physiologic actions are always presented over a spectrum of various signs and symptoms. Tolerance and dependence to different actions, such as alleviation of pain and respiratory depression, develop and disappear independently and on different time scales, causing potentially serious or life threatening complications when dosage is changed.

Based on their origin, opioids can be divided into three main categories: natural and semi-synthetic opiates, synthetic opioids and endogenous opioids. Natural and semi-synthetic opiates share the phenantrene main chemical structure and are further divided into natural opiates that are alkaloids of the poppy plant (Papaver somniferum), and into semi-synthetic opiates that are chemically modified derivatives of the natural opiates. Examples of the natural opiates include morphine, codeine and thebaine. Papaverine and noscapine are also alkaloids of the poppy plant but they are not considered opiates because of their mechanism of action is different from that of the opiate analgesics. Semi-synthetic opiates are produced by chemically modifying side chains on the phenantrene structure and include heroin (di-acetyl morphine), oxycodone, oxymorphone, hydrocodone, hydromorphone, buprenorphine and the opioid antagonist, naloxone among others. Synthetic opioids are derivatives of either of three dissimilar chemical structures, consisting of the benzomorphans (pentazocine and loperamide), phenylpiperidines (meperidine, fentanyl, sufentanil), and diphenylheptanes (methadone and propoxyphene). Endogenous opioids are opioid peptides and include the major groups of endorphins, enkephalins, dynorphins and endomorphines. The clinical laboratory measurement of opioid peptide concentration is not required for pain management at this time therefore we will not discuss further this group of opioids.

Clinical characteristics and metabolism of the opioids most frequently used for chronic pain control

Natural opiates

Morphine and the chemically related opioids produce their major effects through the mu receptors. In addition to analgesia, these effects include nausea, vomiting, drowsiness, respiratory depression, decreased gastrointestinal motility, and antitussive properties. Overdose by these opioids results in stupor, coma, severe respiratory depression, pinpoint pupils and decreased body temperature. Death can result from respiratory depression. Morphine can be administered orally or via s.c., i.m. or i.v. injections. The bioavailability of oral morphine is variable, reported between 15% and 65%, and that of injected morphine is approximately 95% of the dose administered. The half life of morphine in the blood is between 1 and 7 hours, and the administered dose is either metabolized to normorphine, or conjugated with glucuronide at the C-3 or C-6 position. A small amount of administered morphine is excreted unchanged in the urine as free morphine. Free and conjugated morphine can be found together in the blood and urine and if measured together it is referred to as total morphine. Normorphine has limited biological activity and it is only <5% of the total dose therefore does not contribute significantly to the pharmacologic actions of morphine (4). Morphine-3-glucuronide is biologically inactive but morphine-6-glucuronide has considerable analgesic activity (5). Free morphine is more representative of recent administration while conjugated morphine is representative of past morphine use. Free and conjugated morphine can be measured separately to assess patient's compliance with opioid prescriptions or during death investigation of suspected complications of overdose (6).

Therapeutic analgesic doses of morphine vary between individuals, and vary in time within the same individual as tolerance develops. Intravenous or intramuscular injection of 0.125 mg/kg single dose morphine will result approximately 20 ng/mL blood concentration of free morphine 2 to 4 hours after administration and ~75% of the administered dose will be excreted in the urine within 72 hours as conjugated morphine. Serum steady state free morphine concentrations above 20 ng/mL are considered analgesic but large between-individual and within-individual variations exists (4). The daily dose of morphine required to achieve sufficient pain control have been reported between 60 and 1800 mg/day and blood concentrations of morphine in the same group of chronic pain patients have been reported between 16 and 2837 ng/mL (7), (8). The reported daily doses agree with previous reports and can be explained by the development of tolerance. However, the reported blood concentrations must be interpreted with caution because the timing of blood collection in relation to last dose was not controlled and it is not clear from the publication if the assays used were measuring free or total morphine. Other studies reported serum free morphine concentrations above 200 ng/mL to be considered toxic though much lower concentrations (~100 ng/mL) have also been reported in postmortem samples from patients who died of opiate overdose (4).

Codeine is the second most abundant opioid alkaloid of the poppy plant. Chemically it is 3-methyl-morphine. It can be purified from opium or it can be also synthesized from morphine. Its analgesic properties and side effects are very similar to those of morphine while codeine also has very strong antitussive properties. Its metabolism proceeds through N-demethylation to norcodeine (minor pathway) or via 3-demethylation to morphine with a half life of 2-4 hours. The codeine-derived morphine then follows the same metabolic pathways that were described when discussing morphine. Norcodeine is glucuronidated and excreted in urine along with metabolites of the codeine-derived morphine. It is important to remember that codeine is converted to morphine in the human body, but codeine is nor synthesized from morphine physiologically. The previously described trace amount of codeine found in the blood and urine of patients who were treated with morphine are contributed to codeine contamination of the insufficiently purified morphine preparation. Therapeutic and toxic levels of codeine are similar to those of morphine, 10-100 ng/mL and >200 ng/mL, respectively, although the single patient on 120 mg/d codeine in Tennant's publication had 480 ng/mL blood concentration while fully ambulatory and capable of driving motor vehicles (7), (8).

Only codeine is present in the blood immediately after a single dose in a previously opiate naive individual. The concentration of codeine decreases in time as it is metabolized to morphine and the concentration of morphine increases, leading to constantly changing ratio of the two opioids. This change in codeine : morphine concentration ratio can provide a rough estimate of the last dose of codeine administration. The codeine : morphine ratio in urine will remain >1 during the first 24 hours after codeine administration, but then it will "flip", or become <1, between 24-30 hours post dose. Only morphine will be detectable in urine after 30 hours following a single dose of codeine. The timing is somewhat variable between individuals and total morphine and codeine must be measured (after release of the conjugated drug by hydrolysis) in order to observe this change of codeine : morphine ratio (9).

Semi-synthetic opiates

Semi-synthetic opiates are produced from morphine or thebaine by chemically modifying the hydroxyl groups or other parts of the opioid alkaloids. The most commonly prescribed opioids in this group include oxycodone, oxymorphone, hydrocodone, hydromorphone and dihydrocodeine, all chemical variants of morphine. These drugs alleviate pain and induce similar tolerance and side effects as observed with morphine, therefore it should not be surprising to expect large between individual variations in dosing and blood concentrations (Table 1.) The metabolism of oxycodone proceeds through oxymorphone, itself an active

Table 1
Reported lowest and highest daily doses of opioid medications and lowest and highest blood concentrations as observed in chronic pain patients who were fully functional (modified from (8))

Opioid	Previously reported therapeutic range (ng/mL)	Previously reported toxic concentration (ng/mL)	n	Lowest - Highest dose (mg/day)	Lowest - Highest blood concentration (ng/mL)
Codeine	10 - 100	>200	1	120	480
Hydrocodone	8-32	>100	11	50 - 300	18 - 396
Hydromorphone	8-32	>100	11	20 - 540	9.4 - 230
Oxycodone	10 - 100	>200	15	15 - 2700	5 - 3077
Oxycodone (LA)	10 - 100	>200	33	40 - 960	10 - 650
Morphine	10-80	>200	10	100 - 1800	22 - 828
Morphine (LA)	10-80	>200	17	60 - 2000	16 - 2837
Meperidine	70 - 500	>1000	xx		
Normeperidine	50 -280	>8000	xx		
Fentanyl	1 - 3	>8	26	n/a (b)	1.2 - 9.5
Propoxyphene	100 - 400	>500	2	400 - 1300	227 - 240

(LA): denotes sustained release / long acting formulation
(b): all patients received fentanyl via sustained release transdermal or trabsmucosal patches
(xx): not reported in reference (8).

opioid and prescription analgesic. A small amount of the parent drug is converted to noroxycodone. The parent drug and its metabolites will be conjugated to glucuronide and excreted in the urine (4).
Hydrocodone, another semi-synthetic analgesic is produced from codeine. It has stronger analgesic properties than codeine and it also has antitussive properties. It is metabolized to norhydrocodone, hydromorphone and the minor metabolites of hydrocodol and hydromorphole. Approximately 12% of the administered dose is excreted in the urine unchanged within 72 hours. The metabolites will be also conjugated to glucuronide and eliminated via the urine (4).
Heroin, or diacetylmorphine, is not a prescription opiate. It is an illicit street drug with extreme addictive potential that is frequently abused by patient on chronic opioid pain medication. It causes intense euphoria, decreased pain sensation and loss of anxiety in addition to the usual effects of the opioids. It is more lipophilic than morphine therefore it crosses the blood-brain barrier more easily than morphine does. It is converted to 6-monoacetyl-morphine in the central nervous system via deacetylation with a half life of <60 minutes, then further metabolized to morphine. Heroin is most often administered via intravenous route but subcutaneous and oral administration has also been documented in addicts. Because of the very short half life of heroin its first step metabolite, 6-monoacetyl morphine (6-MAM) is the usual target for testing if heroin abuse is suspected. As 6-MAM can't be synthesized by the human body its presence is undeniable proof of heroin use (4).
Figure 1. schematically depicts the major pathways of metabolism of the natural and semi-synthetic opiates. As most of the pathways are unidirectional, as indicated by the arrows, the expected metabolites can be deducted by knowing the prescribed medication and the presence of opiates that can't be produced from the prescribed treatment should indicate illicit use of narcotics.

Synthetic opioids

Meperidine is a phenylpiperidine derivative that is primarily a mu-receptor agonist. It is a weaker analgesic, having about one eight the potency of morphine at equal weight. Its side effects are similar to those of morphine. Meperidine overdose is responsive to naloxone treatment. Meperidine is metabolized in the body to normeperidine that is also an active opioid analgesic. The metabolites of normeperidine are believed to be responsible for the unusual side effects of hyperactivity, muscle twitches, dilated pupils and convulsions; unexpected side effects in opioid overdose. The amount of meperidine and normeperidine in the urine is urinary pH dependent. About 50% of a meperidine dose is excreted in acidic urine as meperidine and normeperidine, but only 5% of the total dose is eliminated in alkaline urine (4).
Fentanyl, another phenylpiperidine and chemical relative of meperidine, has analgesic as well as anesthetic properties. Its analgesic activity is approximately 80-times that of morphine. It elicits strong respiratory depression and a single dose of 0.5 - 1.0 mg fentanyl could be lethal in an opiate-naive individual or in an individual who lost his tolerance. Chemical variants of fentanyl, (e.g. alfentanyl, sufentanyl, carfentanyl) are also available as prescription medications. Fentanyl is lipophilic and accumulates in fatty tissue where it can be released from during rapid weight loss, causing lethal overdose. Fentanyl can be

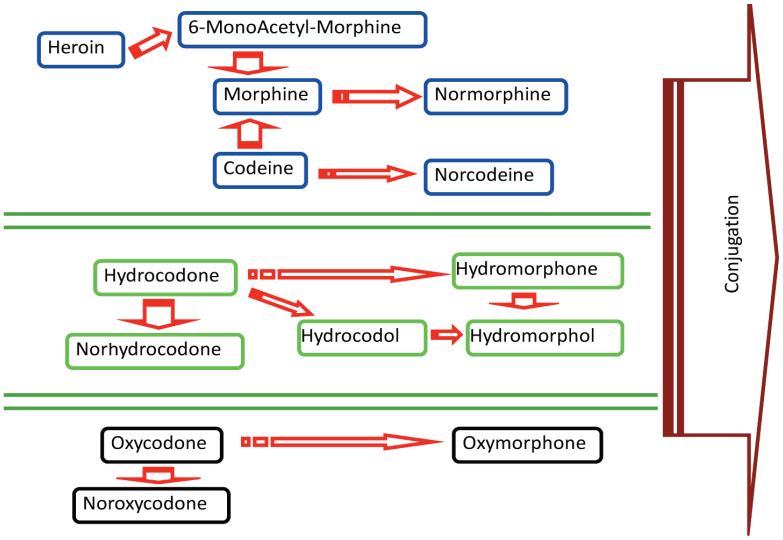

Figure 1
Schematic representation of the most significant pathways of metabolism of natural and semi-synthetic opiates.

administered i.v. or i.m., but because of its short duration of action i.v. drip or transdermal patch are the preferred means of administration. Transdermal patches are manufactured to release 25 to 100 micrograms of fentanyl per hour for management of chronic pain. Patients addicted to fentanyl have been known to place multiple patches on their bodies simultaneously, or to extract fentanyl from patches for intravenous injection (10-13). Fentanyl is metabolized to norfentanyl and both the parent drug and its metabolites can be detected in the urine (4).

Methadone is a diphenylheptane derivative, a long acting opioid that is used both to induce analgesia and to treat opiate withdrawal, although methadone itself will induce tolerance, dependence and addiction with repeated use. Unfortunately, tolerance to methadone is rapidly lost, sometimes after missing just a few doses of the drug, therefore fatal overdoses are not common (14-17). The half life of methadone is variable, reported to be between 4 and 190 hours, although 15-60 hours are more typical (4). In addition to CYP2D6, methadone is also metabolized by CYP3A4 and CYP2B6 enzymes. The heterogeneity of these enzymes in the population leads to "fast" and "slow" metabolizers. Fast metabolizers may not have any parent drug in their blood or urine within just a day or so after the last dose, therefore it is imperative that the methadone metabolites of EDDP and EMDP be measured with the parent drug before non-compliance is concluded on the basis of undetectable methadone concentrations.

Buprenorphine is another synthetic opioid with significant analgesic properties as well as antidepressant activity. It has been also approved by the FDA in the USA for the maintenance therapy of opioid dependent individuals. It is a variant of the phenantrane opiates, therefore it is similar to morphine and the other semi-synthetic opiates, however, it has unique pharmacologic properties. Buprenorphine has extremely long half life therefore it can be administered once every 2-3 days. Discontinuation of the drug will cause 4-5 weeks long "acute", and up to 12 months long "chronic" withdrawal symptoms. Intolerable, withdrawal-related pain sensation has been reported by legitimate users.

Laboratory analysis of opioids and interpretation of results

Both qualitative and quantitative assays have been available commercially for pain medication monitoring. Qualitative assays are designed to detect the presence or absence of a specific opioid thereby assessing patient compliance with prescription. The specimen of choice for qualitative tests is urine, but serum or plasma can also be used as an alternative to urine when the patient is on dialysis. Various manufacturers have developed a) homogenous competitive immunoassay, b) enzyme-multiplied immunoassay technique (EMIT), c) cloned enzyme donor immunoassay and d) kinetic interactions of microparticles in solution (KIMS) immunoassay formats. Other immunoassay formats, such as fluorescence polarization immunoassay (FPIA) or radioimmunoassay (RIA) formulations had been in use in the past. Regardless of the signal-detection system of the immunoassay, the specificity of the test will be dependent on the antibody employed. The various immunoassays have been adapted to large volume laboratory analyzers, but some of the antibodies have been incorporated in point of care test (POCT). For a recent review of the various assay formats and a list of cross reactivity of those assays with various opioids see publication by Reisfield et al. (18).

Quantitative assays are used for therapeutic drug monitoring (TDM) of pain medications but can also be used for the assessment of patient compliance. The most common specimen for quantitative assays are serum or blood.

The urine drug screen immunoassays were originally developed for workplace drug testing and are optimized for detecting the

"NIDA 5" drugs and will give positive reading above the legally mandated cut off. Although opiates were part of the original NIDA 5 group only morphine and codeine cutoffs are regulated. Initially morphine and codeine decision limits were set at 300 ng/mL, but this cutoff concentration was changed to 2,000 ng/mL in 1998 to eliminate the occasional false positives urine screens after consumption of poppy seed containing food. Despite the change in regulatory requirements, urine opiate screening tests are still available at both the 300 ng/mL and the 2,000 ng/mL cut off concentrations and various laboratories may offer urine opiate screening assays at either or both of these detection limits. Unfortunately even the lower, 300 ng/mL cut off is too high for pain medication monitoring, and the 2,000 ng/mL is clearly inadequate for medical purposes.

Another limitation of the urine opiate immunoassays are lack of analytic sensitivity for semi-synthetic opiates. The opiate assays that were designed to screen for the presence of morphine and codeine might be adequate for hydrocodone, hydromorphone and dihydrocodeine detection, but they are unable to reliably measure oxycodone or oxymorphone drug concentrations at therapeutic dosages. For example, both the Syva EMIT and the CEDIA opiate immunoassays will recognize the presence of hydrocodone and hydromorphone at approximately 600 ng/mL drug concentrations, or at twice the 300 ng/mL morphine cut off concentrations. This is often referred to as having approximately "50% cross reactivity" with hydrocodone or hydromorphone. However, the same 300 ng/mL opiate assays will detect oxycodone and oxymorphone only at a much higher, approximately 2,500 ng/mL, concentration. Patients who take oxycodone for chronic pain relief might have urine oxycodone concentrations above the 2,500 ng/mL on occasion, immediately after the last dose, but oxycodone and its metabolites will be present below the 2,500 ng/mL limit most of the time, leading to false negative laboratory result. If these patients are required to demonstrate presence of oxycodone while under treatment they could be mistakenly accused of diverting their medication and could be ejected from the treatment program due to inadequate performance of the screening assay. Synthetic opioids analgesics, such as methadone, naloxone or fentanyl, are not detected at all by the regular urine opiate immunoassays (19).

To eliminate the possibility of false negative opiate test result several manufacturers have developed specific immunoassays for oxycodone/oxymorphone, fentanyl and metabolites, buprenorphine, and methadone and its metabolites. These immunoassays have been marketed by manufacturers of chemistry analyzers under the instrument manufacturers' brand name but reagents are also available from third parties to be used in open channels of the analyzers. POCT devices are also available for detection of individual drugs. Not all of these devices have been evaluated by independent research, therefore laboratories using these devices or reagents must rely on manufacturer's package inserts that may not have very extensive cross reactivity information. At least one large scale study is under way in the United States for the assessment of the accuracy of POCT and in-office drug testing (20).

Conjugation of opioids to glucuronide and sulfate is the main mechanism of their deactivation and elimination from the body. As discussed previously, conjugation can occur at both the 3- and 6-carbon position of the morphine analogs, leading to mostly inactive metabolites. As manufacturers of the various immunoassays don't always report their assay crossreactivity with the conjugates it is often times difficult to assess the contribution of opioid metabolites to the final result, and different immunoassays can arrive to different conclusion regarding the presence or absence of opioids in the same specimen (21). For example, both the MO-3 and MO-6 glucuronide conjugates are recognized at ~60% cross reactivity by the CEDIA opiate immunoassay but the Syva EMIT assay package insert does not provide information regarding glucuronide conjugate cross reactivity. As most laboratories don't publish the immunoassay brand they use for opiate testing nor the cross reactivity of their assays with the various opiate metabolites the clinician is left in the dark regarding test result interpretation.

Metabolism of the synthetic opioids can also pose a challenge for laboratories that perform testing for pain medication. Fentanyl, with it's very short half life might not be detected by screening assays if the patient is not using sustained release formulation, therefore fentanyl monitoring assays must be able to detect norfentanyl as well. Methadone is another opioid that may be reported as false negative due to between-individual differences in metabolism (22). The measurement of the major metabolite of EDDP is, therefore, mandatory if the patient on methadone maintenance therapy can lose his prescription after a false negative laboratory result. Ideally methadone screening assay should measure the parent drug, EDDP and the minor metabolite, EMDP.

Considering the complexity of the chemistry and metabolism of the various opioids and the limitations of the opioid immunoassays, it should not be surprising that more specific testing methods were required by clinicians and laboratorians. Simultaneous detection of the chemically dissimilar opioids, at a sufficiently low cutoff concentration, with the ability to measure free and conjugated parent drugs and their metabolites required new technology. Chromatographic separation of the analytes and detection by mass spectrometry seemed to be an ideal candidate for this type of testing. Numerous analytic methods were published using gas chromatography - mass spectrometry (GC-MS) (23-25). Only a few clinical laboratories had the resources to implement and validate published methods or to develop their own. GC-MS based methods have also been used for confirmation of positive opioid screening assays.

The rapid development in atmospheric pressure ionization mass spectrometry technology allowed the coupling of liquid chromatography to single quadrupole mass spectrometers and then to tandem mass spectrometers. More recently Time of Flight (TOF) mass spectrometers have also been used for rapid identification of unknown drugs in serum or urine. The advantage of this technology, often referred to as LC-MSMS, is the simple and fast sample preparation, no requirement for sample derivatization, short sample-to-sample injection times, and the possibility of screening and quantitation of the detected opioids

from the same sample. Reference laboratories and a few clinical labs have developed proprietary analytic methods for the simultaneous measurement of the common opioids and their metabolites but published methods are also available in the literature (26, 27). Recently published methods can screen for over 30 opioids within a 10 minute run and offer lower limit of detection (LLOD) around the 5 to 10 ng/mL concentration range for most opioids. The disadvantages of LC-MSMS technology are very high initial acquisition cost of the equipment, lack of experienced operators in the clinical laboratories and the requirement for developing, validating and maintaining laboratory developed tests (LDTs).
Even when analytic techniques are appropriate to detect the presence of multiple parent drugs and metabolites, care providers have difficulty interpreting laboratory results of opioid measurement. A study by Levy et al. (28) surveyed 359 physicians who routinely ordered and interpreted urine drug results regarding collection, use and interpretation of urinary opioid test results. Only 10% of the respondents answered all survey questions correctly and 75% of the ordering physicians answered one or more items incorrectly. A recently published practice guideline by Peppin et al. attempts to provide framework for ordering, analyzing and interpreting opioid test results (29). While these recommendations were written by clinicians for clinical practitioner, we, laboratory professionals, should also participate in the conversation. As clinical laboratory directors, we must have the understanding of the analytic principles of opioid testing and we must provide technical guidance and interpretations of results in help of our clinical colleagues.

Conclusions

Analgesic opioids are a chemically diverse group of pain medications that undergo complex metabolism leading to a number of opioid-like chemicals in the body even after treatment with a single agent. The great addictive potential of opioid drugs requires close monitoring of the use of prescribed medications and of illicit opioids.
Traditional opiate immunoassays were not designed for medical use. Their analytic sensitivity is insufficient to unambiguously detect the presence of the prescribed opioid in biological fluids. In order to assess the concurrent use of prescribed and illicit opioid use (e.g.: heroin use by a patient who has methadone prescription) multiple immunoassays must be ordered and the results of those assays must be interpreted in context.
Chromatography - mass spectrometry based screening and confirmation assays are becoming more common due to their ability to detect many or most prescription pain medications and their metabolites from a single specimen at clinically meaningful low concentrations but only a few clinical laboratories have the financial resources and technical expertise to offer these methods. The rapid acceptance of LC-MSMS analytic techniques are also hindered by the extra burden of developing and validating methods in house.
Finally, not all clinicians are fully aware of the complexities of opioid drug testing. The clinical laboratories must be more proactive informing the clinicians about the specificity, sensitivity and interferences of the laboratory's opioid assays and we need to provide active help with ordering the right test for the situation and interpreting the results.

References

1. Schappert, S.M. and C.W. Burt, Ambulatory care visits to physician offices, hospital outpatient departments, and emergency departments: United States, 2001-02. Vital Health Stat 13, 2006(159): p. 1-66.
2. The management of chronic pain in patients with breast cancer. The Steering Committee on Clinical Practice Guidelines for the Care and Treatment of Breast Cancer. Canadian Society of Palliative Care Physicians. Canadian Association of Radiation Oncologists. CMAJ, 1998. 158 Suppl 3: p. S71-81.
3. Ives, T.J., et al., Predictors of opioid misuse in patients with chronic pain: a prospective cohort study. BMC Health Serv Res, 2006. 6: p. 46.
4. Randall C. Baselt, P.D., Disposition of Toxic Drugs and Chemicals in Man. Eighth Edition ed: AtlasBooks.
5. Osborne, P.B., B. Chieng, and M.J. Christie, Morphine-6 beta-glucuronide has a higher efficacy than morphine as a mu-opioid receptor agonist in the rat locus coeruleus. Br J Pharmacol, 2000. 131(7): p. 1422-8.
6. Jones, A.W., A. Holmgren, and J. Ahlner, Concentrations of free-morphine in peripheral blood after recent use of heroin in overdose deaths and in apprehended drivers. Forensic Sci Int, 2012. 215(1-3): p. 18-24.
7. Tennant, F., Opioid serum concentrations in patients with chronic pain. J Palliat Med, 2007. 10(6): p. 1253-5.
8. Tennant, F., Opioid blood levels in high dose, chronic pain patients. Practical Pain Management, 2006: p. 1-8.
9. Cone, E.J., et al., Forensic drug testing for opiates, III. Urinary excretion rates of morphine and codeine following codeine administration. J Anal Toxicol, 1991. 15(4): p. 161-6.
10. Tharp, A.M., R.E. Winecker, and D.C. Winston, Fatal intravenous fentanyl abuse: four cases involving extraction of fentanyl from transdermal patches. Am J Forensic Med Pathol, 2004. 25(2): p. 178-81.
11. Edinboro, L.E., et al., Fatal fentanyl intoxication following excessive transdermal application. J Forensic Sci, 1997. 42(4): p. 741-3.
12. Flannagan, L.M., J.D. Butts, and W.H. Anderson, Fentanyl patches left on dead bodies -- potential source of drug for abusers. J Forensic Sci, 1996. 41(2): p. 320-1.
13. Chaturvedi, A.K., N.G. Rao, and J.R. Baird, A death due to self-administered fentanyl. J Anal Toxicol, 1990. 14(6): p. 385-7.
14. Rosca, P., et al., Mortality and causes of death among users of methadone maintenance treatment in Israel, 1999-2008. Drug Alcohol Depend, 2012.
15. Jones, A.W., A. Holmgren, and J. Ahlner, Blood methadone concentrations in living and deceased persons: variations over time, subject

demographics, and relevance of coingested drugs. J Anal Toxicol, 2012. 36(1): p. 12-8.
16. Madden, M.E. and S.L. Shapiro, The methadone epidemic: methadone-related deaths on the rise in Vermont. Am J Forensic Med Pathol, 2011. 32(2): p. 131-5.
17. Leach, D. and P. Oliver, Drug-related death following release from prison: a brief review of the literature with recommendations for practice. Curr Drug Abuse Rev, 2011. 4(4): p. 292-7.
18. Reisfield, G.M., et al., Urine drug test interpretation: what do physicians know? J Opioid Manag, 2007. 3(2): p. 80-6.
19. Reisfield, G.M. and G.R. Wilson, Rational use of sublingual opioids in palliative medicine. J Palliat Med, 2007. 10(2): p. 465-75.
20. Manchikanti, L., et al., Protocol for accuracy of point of care (POC) or in-office urine drug testing (immunoassay) in chronic pain patients: a prospective analysis of immunoassay and liquid chromatography tandem mass spectometry (LC/MS/MS). Pain Physician, 2010. 13(1): p. E1-E22.
21. Reisfield, G.M., E. Salazar, and R.L. Bertholf, Rational use and interpretation of urine drug testing in chronic opioid therapy. Ann Clin Lab Sci, 2007. 37(4): p. 301-14.
22. Leimanis, E., et al., Evaluating the relationship of methadone concentrations and EDDP formation in chronic pain patients. J Anal Toxicol, 2012. 36(4): p. 239-49.
23. Nowatzke, W., et al., Distinction among eight opiate drugs in urine by gas chromatography-mass spectrometry. J Pharm Biomed Anal, 1999. 20(5): p. 815-28.
24. Smith, M.L., et al., Forensic drug testing for opiates. VI. Urine testing for hydromorphone, hydrocodone, oxymorphone, and oxycodone with commercial opiate immunoassays and gas chromatography-mass spectrometry. J Anal Toxicol, 1995. 19(1): p. 18-26.
25. Broussard, L.A., et al., Simultaneous identification and quantitation of codeine, morphine, hydrocodone, and hydromorphone in urine as trimethylsilyl and oxime derivatives by gas chromatography-mass spectrometry. Clin Chem, 1997. 43(6 Pt 1): p. 1029-32.
26. Shakleya, D.M., et al., Simultaneous liquid chromatography-mass spectrometry quantification of urinary opiates, cocaine, and metabolites in opiate-dependent pregnant women in methadone-maintenance treatment. J Anal Toxicol, 2010. 34(1): p. 17-25.
27. Fox, E.J., S. Twigger, and K.R. Allen, Criteria for opiate identification using liquid chromatography linked to tandem mass spectrometry: problems in routine practice. Ann Clin Biochem, 2009. 46(Pt 1): p. 50-7.
28. Levy, S., et al., Drug testing of adolescents in ambulatory medicine: physician practices and knowledge. Arch Pediatr Adolesc Med, 2006. 160(2): p. 146-50.
29. Peppin, J.F., et al., Recommendations for Urine Drug Monitoring as a Component of Opioid Therapy in the Treatment of Chronic Pain. Pain Med, 2012.