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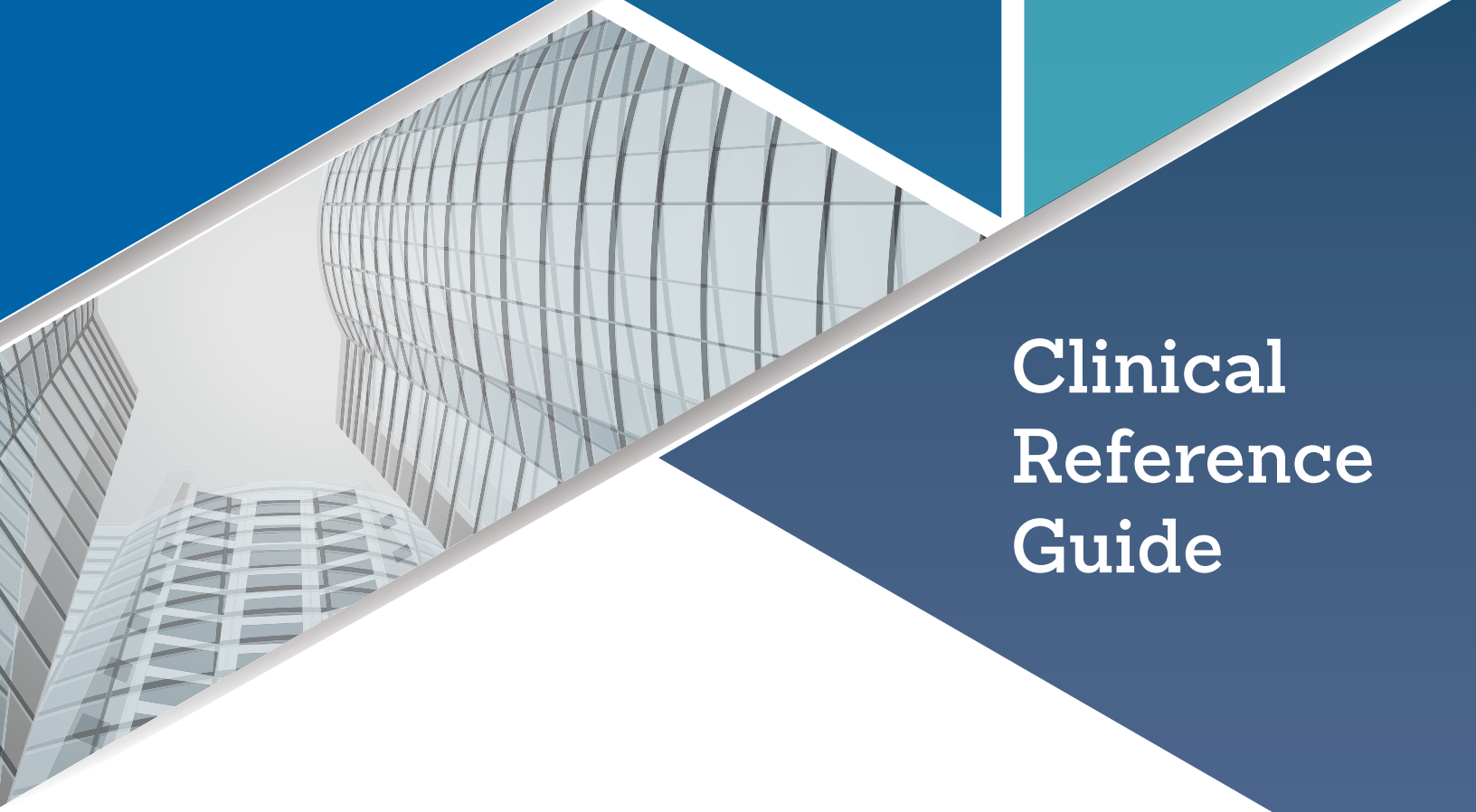

Clinical Reference Guide

Methodology Used for Presumptive and Definitive Medication Adherence Testing

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Testing directly by gas chromatography/mass spectrometry (GC/MS) or liquid chromatography/tandem mass spectrometry (LC/MS/MS) may prevent false positives and negatives through identification of drugs and metabolites undetected by presumptive methods.

Immunoassay is the most common method used for presumptive drug testing and is frequently employed by on-site/point-of-care testing (POCT) in outpatient clinics and hospital laboratories. The most frequently used definitive methods include gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS). Clinicians should note that not all LC/MS/MS methods meet criteria to be considered definitive; depending on how LC/MS/MS is performed, it may also be used as a presumptive method.

A. Immunoassay

Immunoassay is based on the principle of competitive binding of an antibody to a target analyte (drug or drug metabolite). If a different drug is similar in structure to the target analyte, it may bind to the antibody and trigger a positive result. However, some drugs with no clear structural similarity to the target analyte may still bind to the antibody. These cross-reacting compounds may result in false positives; alternatively, lack of cross-reactivity across a class may result in false negatives.[1-3] The extent of cross-reactivity across drugs in a class (or to other cross-reacting compounds) may vary depending on the immunoassay used.

Presumptive testing by immunoassay does not provide a concentration of a specific drug, but rather qualitative results for a drug class (i.e., present or not present). Semi-quantitative results may be obtained by certain types of immunoassay, but these results are not exact (or definitive) because they do not represent a specific drug and may include other cross-reacting compounds. Relying solely on immunoassay results is problematic for adherence testing purposes because the identification of a drug class does not demonstrate adherence with prescribed drug treatment. For example, an opiate class positive does not distinguish between use of morphine or heroin, or the use of multiple opiates versus one opiate. The primary advantage of immunoassay is that it yields a rapid result. For this reason, it is often used on-site when an immediate result is desired, despite the sacrifice in accuracy.[1]

B. Definitive Methods

GC/MS and LC/MS/MS are just two of the myriad of ways that scientists can identify drugs and their related metabolites. Although definitive methods need not be limited to these, both are frequently used for definitive analyses for urine drug testing in pain management.

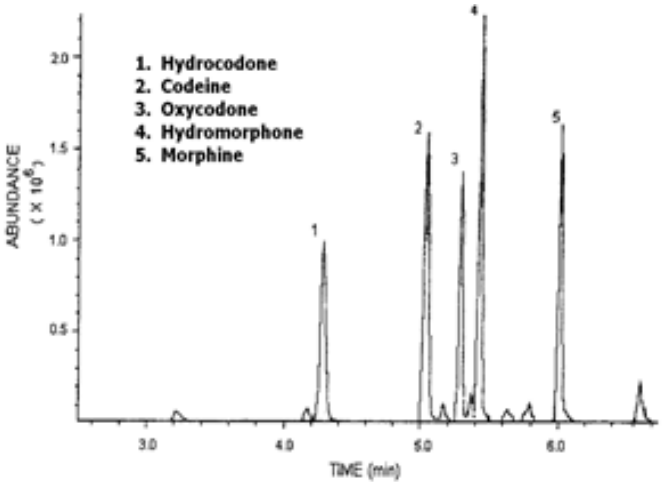
The purpose of chromatography (whether by gas or liquid) is to separate compounds by a partitioning process. This separation will allow for the individual identification of unique drugs and metabolites by a detector (e.g., mass spectrometry).[4] Chromatographic separation of drugs and metabolites occurs because these compounds are chemically unique and interact differently in the GC or LC column. Some compounds elute (separate) faster on the column than others, yielding peaks with different retention times (see Figure 2.1). Subsequently, each drug or metabolite present will yield a peak at its expected retention time; successful compound separation will ensure that these peaks do not overlap.

Chromatographic resolution relates to the distance between two peaks, or more accurately put, the differences in their retention times. In general, the higher the resolution, the more distance between peaks. If resolution is low and peaks begin to overlap, it may become difficult to tell analytes apart from one another.

Resolution, sensitivity, and specificity also apply to mass spectrometry. Mass spectrometers have an innate ability to “resolve” drugs and metabolites based on their chemical composition and mass. Increased mass

resolution results in an improved ability to uniquely identify a drug or metabolite (specificity). As mass resolution and specificity improve, sensitivity also improves.[4]

Figure 2.1: Chromatography and Mass Spectra for Opiates



GC/MS vs. LC/MS/MS...Which is Better?

GC/MS, due to its reliability and accuracy, has long been called the "gold standard" of drug testing and has been used for Federal Workplace Drug Testing programs for many years.[5] GC/MS and LC/MS/MS are both useful tools to keep in the toxicologist's arsenal, and method selection should depend on the chemical properties of the compound(s) to be analyzed. Both methods offer advantages as well as limitations. Gas chromatography methods are most effective for nonpolar, volatile chemicals, while liquid chromatography is preferable for polar and nonvolatile compounds.[4,6-8] Although more compounds are amenable to testing by LC, GC provides greater separating power but typically requires more sample volume. Compounds analyzed by GC/MS methods may also require derivatization to increase volatility; this step increases both the time and cost of sample preparation. LC/MS/MS methods may require less sample preparation on the front end, and "dilute and shoot" methods can greatly simplify the testing process. In addition, tandem MS analysis intrinsic to LC/MS/MS improves selectivity and can overcome minimal sample preparation and limited chromatographic separation. While LC/MS/MS is an extremely powerful tool for the analysis of drugs in biological samples, it is not without some significant limitations. Co-eluting (insufficiently separated) compounds may be underestimated or not detected at all.[6] Interferences may confound accuracy, especially when drug concentrations are either very low or very high; for example, some metabolites of oxycodone can interfere with the analysis of structurally similar opiates like dihydrocodeine.[9] In addition, the O-desmethyl-venlafaxine metabolite of venlafaxine (Effexor®) has been described to be misidentified as tramadol because the two share transitions on some LC/MS/MS methods.[10] Consequently, when a large number of compounds that are structurally and chemically similar are analyzed (e.g., opiates and related metabolites), the testing method requires greater separation of compounds for reliable results.[6,9]

One extremely important consideration is a phenomenon known as ion suppression. When substances exit the chromatography column, they enter the ion source.[11] If a lot of drug and/or matrix elute at the same time, there may not be enough electrons available to ionize all drug present; this is ion suppression. This can lead to low, or even absent, signal for that drug and a false negative result.[11,12] Methods must be subjected to rigorous evaluation in order to minimize the risks of ion suppression. While extensive use of stable isotope internal standards is one way to compensate for ion suppression,[13] it is not a universal solution for this problem. Analysts with extensive experience in observing this phenomenon are best prepared to reanalyze a sample with reduced matrix to optimize recovery and acheive accurate results.

Some laboratories seek to exploit the resolving power of tandem mass spectrometry by employing minimal sample preparation techniques. Such "Dilute and Shoot" methods are very fast and cost efficient.[11] Use of this method can be handicapped by the very complex nature of urine specimens and the relatively limited chromatographic resolving power of liquid chromatography columns. This method is particularly impacted by ion suppression as the sample is not cleaned-up prior to analysis.[14]

Many of the compounds in pain management are accurately identified and measured by LC/MS/MS. Opioids and benzodiazepines are, in particular, highly amenable to analysis by LC/MS/MS, and several methods have been described in the literature.[2,9,15-17] However, GC analysis offers a few advantages over LC analysis, such as identification of a different variety of compounds

(including those that may escape detection by LC/MS/MS), lower detection limits for some drugs, improved compound separation, and significant elimination of ion suppression or chromatographic interferences.[7,18,19] Some drug classes that are routinely and successfully analyzed by GC/MS include: barbiturates, marijuana, amphetamines, and steroids.[4,6] While LC/MS/MS methods do exist for some of these drugs and drug classes, they may not be advantageous over GC and may have analytical limitations.

Supporting both technologies (GC/MS and LC/MS/MS) is expensive and beyond the financial and technical reach of many laboratories. When only one type of analytical system is available, methods must be forced to work on that chromatographic platform, and sacrifices in analytical quality may result.[8]

Are All Methods Alike?

Not all GC/MS or LC/MS/MS methods are equivalent. Methods are designed to optimize the conditions of analysis (temperature, pressure, pH, etc.) to allow for complete compound separation. Typically, a validated chromatography method is the best compromise to achieve effective resolution for each compound in an analysis. Development of an effective chromatographic method is slow, expensive, and difficult. Each time a chromatographic method is optimized for one analyte, separation of all the others becomes less optimized, and resolution between peaks may decrease. The quality of chromatography is inversely proportional to the number of analytes included in a single method.[4]

Because LC/MS/MS analytical methods are often faster than GC/MS, some laboratories have adopted LC/MS/MS as their only technology. Furthermore, some laboratories specializing in pain management are using one analysis to detect everything in their profile. This abbreviated method is relatively fast and supports a 24 to 48 hour turn-around-time but may fail to achieve the desired level of accuracy, resulting in a higher incidence of reporting errors. More specific methods should be used to determine the presence of each drug if such an LC/MS/MS method is used, which fits the functional definition of a presumptive test rather than true definitive testing.

Analytically, performing analysis of all compounds in one LC/MS/MS analysis may result in the following:

- As resolution decreases and chromatographic peaks begin to overlap, the ability of the mass spectrometer to uniquely distinguish one analyte from another decreases.
- Peaks do not achieve a Gaussian (bell) shape but may begin to tail, possibly affecting the accuracy of the quantitation.
- Compounds may not separate sufficiently and may escape detection altogether. Matrix varies from sample to sample, and interferences can cause false negative results.

Clinically, this can result in the misidentification of a compound (false positive) or failure to report a compound (false negative).

How Can a Non-toxicologist Know Whether a Laboratory's Testing Methods are Appropriate?

While there are criteria which may be used by a clinician to evaluate a laboratory's approach to testing, there is no single reference to use for developing a testing method or judging its accuracy. Many researchers have published details of their methods in peer reviewed literature, but laboratory-developed methods are typically considered proprietary and the details may not be published. A laboratory should perform extensive validation, undergo numerous proficiency studies with various accrediting organizations, and submit to inspections by a number of regulatory bodies.

The GC/MS and LC/MS/MS methods used at Aegis have been designed through extensive research and in-house validations to ensure that they have the optimum combination of chromatographic and mass spectral resolution, with the specificity and sensitivity to ensure the most accurate and reliable testing results. When LC/MS/MS is used as a presumptive method, we use an additional, more definitive LC/MS/MS analysis.

C. Recommendations for Medication Adherence Testing

Testing with a definitive method is required to assess

adherence with a specific prescribed drug.[1] It is imperative to note that positive immunoassay results are presumptive, due to the risk of false positives. Misinterpretation of immunoassay results may be costly and detrimental to patient care. In addition, presumptive testing results may not hold up if challenged in a legal setting.[20-22]

Definitive testing provides the option for the quantitative measurement of drug concentrations in urine. Though certain interpretation limitations exist, quantitative urine drug testing offers interpretive value for the following scenarios.

Drug Adulteration

While excretion of some parent drug in absence of metabolites may occur promptly after ingestion, it is rarely observed in urine for opiates such as hydrocodone and oxycodone.[23,24] Pharmacogenetic poor metabolizer status or drug-drug interactions could increase the risk of impaired metabolism, leading to a parent-drug-only finding in urine in limited cases. This scenario also depends on the number of metabolic pathways represented by inclusion of various drug metabolites in testing. When parent drug concentrations are high and/or multiple metabolites are absent, a possibility exists that the patient added drug directly to the urine specimen. Patients who attempt to appear adherent with prescribed medications may resort to this tactic to avoid an otherwise aberrant negative finding.[25] Although post-collection adulteration with prescribed drugs may be associated with low concentrations if patients are technically knowledgeable, high concentrations should serve as a red flag. A review of nearly 40,000 urine specimens taken from pain management patients indicated that 2.9% exhibited parent drugs in absence of metabolites; more than half exhibited parent-drug-only concentrations over the median concentration observed for that drug in urine.[26] These cases represent a high probability of adulteration.

High Concentrations/Statistical Outliers

Drug concentrations that exceed the normal distribution for drug disposition in urine may be suggestive of potential abuse or misuse.[1] If a drug concentration is a true statistical outlier (e.g., in the top 2.5% or 1% of measured results), careful follow-up with the patient is warranted.

Assessing Potential Source of Positive Findings from Opioid Metabolism

Codeine, hydrocodone, and oxycodone are metabolized to other pharmaceutically-available drugs (morphine, hydromorphone/dihydrocodeine, and oxymorphone, respectively). Additionally, minor metabolism routes may contribute to hydromorphone presence from morphine metabolism, and hydrocodone from codeine metabolism. In such cases, knowing the relative concentrations of parent/metabolite can help determine whether such an analyte could be present due to metabolism versus exogenous ingestion of a separate opioid. Although metabolism and excretion patterns will vary dependent on the individual patient, in the cases of minor metabolism, hydrocodone and hydromorphone should typically fall under 5 and 6% of the parent drug concentrations, respectively.[27-32]

Assessing for Potential Incidental Exposures

Patients undergoing drug testing may occasionally test positive for pharmaceutical impurities in conjunction with the prescribed medication(s). This is a well documented problem for patients prescribed opiates. Selected known pharmaceutical impurities include hydrocodone in oxycodone, oxycodone in oxymorphone, and codeine in morphine preparations.[33-36] Assessing the relative concentrations of unexpected opiate positives is required to prevent inappropriate accusations of drug misuse.

Codeine and morphine may result from ingestion of poppy seeds, though concentrations are typically low (<2,000 ng/mL).[37,38] Quantitative results may serve as an important deciding point between a benign positive result due to ingestion of various foodstuffs and potential non-adherence involving a nonprescribed opioid.

Low concentrations of alcohol metabolites (ethyl glucuronide and ethyl sulfate) or nicotine metabolite (cotinine, 3-hydroxycotinine) may result from incidental exposure.[22,39] Although thresholds employed by Aegis are designed to rule out most cases of incidental exposure, positive findings at or near threshold

may be open to interpretation when heavy passive exposure is reported by the patient. Knowing whether a concentration is high can help interpret a patient's claim of incidental exposure/ denial of use.

Assessing Reuse of Illicit Drugs Using Concentrations Normalized by Creatinine

During addiction treatment, clinicians may wish to observe declining concentrations over time to assess for reuse.[22] This approach has traditionally been reserved for drugs with long detection periods, such as marijuana.[40,41] Such evaluations are only possible with quantitative information.

Assessing for Impact of Potential Drug-Drug Interactions

If relative ratios of parent to metabolites are unusual, clinicians may wish to assess for potential drug-drug interactions or obtain pharmacogenetic information.[22] Poor or ultrarapid metabolizer status may increase the risk of treatment failure or toxicity for drugs such as codeine and perhaps tramadol.[42] Likewise, enhancement of a specific metabolic pathway-as can occur with cytochrome P450 (CYP450) 3A4 inducers-may reduce pain control with opioids, as CYP3A4 metabolites are typically less active than parent compounds and do not contribute substantially to analgesia.[43-45]

A published analysis of 35,000 urine specimens from pain patients evaluated for four of the above scenarios (e.g., parent drug in absence of metabolites, statistically high concentrations, minor metabolism, and pharmaceutical impurities) indicated that 16% required quantitative results to allow interpretation.[46] The potential need for quantitative results has been identified by the American Society of Addiction Medicine, the American Association for Clinical Chemistry, and independent researchers at Brigham and Women's Hospital/ Harvard Medical School; the latter investigators, during optimization of their drug testing paradigm, reported changing reference laboratories specifically to ensure provision of quantitative results.[22,47,48] Aegis provides quantitative testing in pain medication adherence testing and has incorporated many of these interpretive considerations into the report format to streamline utilization for healthcare providers. Without such reporting rules in place, providers must be aware of all of the potential interpretation challenges (e.g., pharmaceutical impurities, minor metabolic pathways). As interpretative knowledge is rapidly evolving in the analytical toxicology field, it would be onerous and potentially unrealistic for all healthcare providers to remain abreast of scientific developments. Provision of quantitative results and interpretive assistance by laboratory experts is imperative to ensure quality patient care.

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