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Mini-Review

Bridging the gap: The critical role of laboratory developed tests in clinical toxicology

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Clinical toxicology is a sub-specialty of laboratory medicine that involves the evaluation of body fluids to identify chemicals, drugs, or toxins [1]. This testing can provide clinicians with valuable information for diagnosis and management of their patients. It can be qualitative or quantitative and used in a wide range of clinical scenarios, including verifying prescription compliance, detecting toxicity, and identifying inappropriate drug use. Although therapeutic drug monitoring (TDM) and medicolegal testing are often associated with clinical toxicology, they deserve their own discussion and are not addressed in this mini-review article. Here, we focus on the utility of laboratory developed tests (LDT) in clinical toxicology.

1. In vitro diagnostics and laboratory developed tests in clinical toxicology

For the majority of general laboratory testing, commercial in vitro diagnostic (IVD) assays are available and regulated by the United States Food and Drug Administration (FDA) as medical devices. Laboratories must abide by the manufacturer's instructions for use, including how the assay is used clinically and which sample types are tested (e.g., serum, urine, etc.) [2]. This rigidity preserves the assay's performance characteristics as defined by the assay manufacturer. In some cases, an FDA-cleared/approved test may not be able to meet clinical needs, in which case a qualified laboratory can employ an LDT that bridges the gap in clinical care or improves clinical workflow [3]. Also, an LDT may be more cost-effective than an IVD for smaller testing volumes.

The Clinical Laboratory Improvement Amendments (CLIA) allow laboratories to create LDTs in several ways, such as modifying an FDA-cleared/approved IVD test (e.g., testing a sample type not listed in the product insert), implementing a protocol for a test developed as an LDT by another institution, or creating a completely new test [2]. For any LDT, the performing laboratory is required to assess its performance characteristics, which would otherwise be determined by the IVD manufacturer, and verify its suitability for patient care [2]. LDTs are categorized as high complexity, meaning that laboratories must meet additional personnel, proficiency testing, and quality specifications [2]. Although laboratories may prefer to utilize IVD assays when available and feasible to avoid the effort associated with LDTs, these tests are essential for responding to evolving clinical testing needs.

The potential for legislative reform to expand oversight of LDTs has raised concerns within the laboratory medicine community. The proposed regulatory framework could make the implementation of LDTs cost-prohibitive and impede laboratories' ability to respond quickly to new needs in patient care [4]. Should this reform be enacted, the clinical toxicology landscape could change drastically, likely having a negative impact on laboratories' ability to provide cutting-edge clinical toxicology testing.

IVD assays play a critical role in clinical toxicology, as they are commonly used as first-line screening tests for the presence of drugs and toxic substances. However, they have several limitations that are particularly relevant in the clinical toxicology space. The vast majority of toxicology IVDs are immunoassays, which typically suffer from

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insufficient detection sensitivity or poor specificity for the intended drug or toxin [5]. Though several mass spectrometry (MS)-based toxicology IVD assays are available in Europe [6], they are currently limited to TDM applications in the United States [7,8]. Additionally, for all but the most common drugs and toxins, IVD assays may simply not be commercially available. Despite having been a cornerstone of clinical toxicology testing for decades, IVD assays are unable to meet all clinical needs at this time. LDTs address the shortcomings of IVD assays and provide laboratories with the testing support necessary to properly assess toxicology patients.

A comprehensive database of all LDTs in clinical use does not exist, however, data from literature and proficiency testing surveys suggest that chromatographic methods such as gas or liquid chromatography (GC, LC) coupled to MS and high-resolution MS (HRMS) are the most prevalent LDTs in clinical toxicology. They are generally superior to IVD immunoassays in their analytic performance [9]. Though there are LDTs available utilizing other testing methodologies, MS-based LDTs remain dominant in clinical toxicology and have significantly increased in popularity over the past decade [4,6,10]. In 2022, the College of American Pathology (CAP) 'Drug Monitoring for Pain Management' (DMPM) proficiency testing surveys had around 500 participants, with approximately 80% generating quantitative results and 15% generating qualitative results by chromatographic methods (i.e., LCMS and HPLC). This demonstrates the extent of integration of MS-based LDTs in clinical toxicology. The following section discusses the known gaps between existing toxicology IVD assays and clinical needs, as well as examples of how LDTs have been used to address them.

2. LDTs are bridging the gaps between patient needs and available toxicology IVDs

2.1. Controlled substance monitoring

Toxicology testing can be used to gauge compliance with a controlled substance prescription regimen, which can mitigate risk for both patient and healthcare provider by identifying the presence of the prescribed controlled substance, the absence of non-prescribed medications, and the absence of illicit drugs. Drug testing for this clinical indication is most commonly performed with a two-step process that begins with an initial qualitative drug screen via immunoassay, followed by a confirmatory test for specific drugs or drug classes that screened positive. Initial screening is typically accomplished with IVD immunoassays, though LDT immunoassays can be used if an IVD solution is not available. Qualitative toxicology screening using MS-based LDT methods is also possible, though not as commonly used [11]. These screening results are usually reported as "presumptive" or "preliminary" positive and are confirmed by a more advanced analytical method to reduce the risk of false-positive results. Quantitative MS-based LDTs have traditionally been used for confirmatory or "definitive" testing due to their superior sensitivity and specificity. Notably, no FDA-cleared confirmatory assays exist, despite MS LDTs being considered the gold standard for confirmatory testing and having been a mainstay of clinical drug testing for decades.

IVD immunoassays offer several advantages, such as ease of use due to being highly automated, quick result turnaround times, and relatively low cost. However, they also come with several limitations that make assessing prescription compliance challenging. For instance, drugs of abuse immunoassays are susceptible to false-positive results by cross-reacting with compounds that are structurally or chemically similar to their target, such as some over-the-counter nasal decongestants or the prescription medication bupropion with many IVD amphetamine drug screens [12,13]. To determine whether the initial screening result was a true or false positive, confirmatory testing using an MS LDT is necessary. This provides the clinician with additional confidence when deciding to either refill or discontinue a prescription. Additionally, many qualitative IVD immunoassays are designed to detect an entire class of drugs, so a positive test result does not indicate which specific compound(s) are present in the sample. MS LDTs, however, can provide identification of the specific drugs and metabolites present, along with their relative concentrations, which can help determine prescription compliance and the potential abuse of multiple drugs within a class [14]. Currently, toxicology MS LDTs provide clinicians with important information that IVD methods cannot.

Immunoassays are prone to false-negative results due to the higher cutoffs for positivity set by IVD manufacturers compared to MS-based LDTs [12,15]. In clinical settings, cutoffs for many IVD drugs of abuse are an order of magnitude higher than the cutoff for MS-based LDTs [14,16]. For example, the common cutoff for benzodiazepine immunoassays in clinical laboratories is 200 ng/mL, while MS-based LDTs can easily quantitate levels $\leq$ 20 ng/mL [17]. The traditional screening with reflex approach to drug testing minimizes false-positive results, but it is not ideal for scenarios with high positivity rates like medication adherence monitoring, which require a testing approach that reduces false-negative results. MS-based LDTs can accurately detect lower concentrations of the drug/metabolite [15,18], providing a longer window of detection, and thus significantly reducing the number of false-negative results.

Immunoassays designed to screen for an entire drug class are calibrated with a representative compound and have varying cross-reactivity with the different drugs and metabolites in that class. As a result, some drugs require higher concentrations to be detected, while others may not be detected at all. Opiate assays are a prime example, since they are designed to have good cross-reactivity with the natural opiates (e.g., morphine, codeine), but have lower cross-reactivity with semi-synthetic opioids (e.g., oxycodone) and hardly any with completely synthetic opioids (e.g., tramadol, fentanyl). For this reason, clinical laboratories often offer separate, more specific immunoassay drug screens to detect commonly-prescribed and abused semi-synthetic and synthetic opioids. Benzodiazepine assays are also a good example due to the high number of benzodiazepines and their extensive metabolism. Commonly-prescribed benzodiazepines such as clonazepam and alprazolam are often missed by immunoassay screens because they cannot reliably detect the metabolites, which are the relevant forms of the drug, given only a small percentage of the drug is eliminated as the parent or unchanged [19,20]. MS-based drug testing methods are more reliable for detecting drugs within a class, as they target each compound specifically, and can distinguish between parent compounds and metabolites. Therefore, they are more suitable for evaluating medication adherence [16,19,20]. Though there are differences in the lower limits of quantitation between compounds in MS-based methods, they are far less pronounced than in immunoassays and less likely to affect clinical decisions.

Given the advantages that MS-based toxicology techniques offer over immunoassays, many have advocated for their use as the first line testing methodology, particularly in the pain management setting for frequently prescribed drugs such as opioids and benzodiazepines [11,21]. Some have adopted a hybrid approach, combining the strengths of each technology and performing MS-based testing for select drugs in parallel with immunoassays screening for others, based on assay performance and anticipated positivity rates [22,23]. The literature demonstrates that toxicology LDTs undeniably improve the quality of results and patient care in this population, regardless of whether the traditional or hybrid approach is used.

2.2. Addiction medicine

In addiction medicine, drug testing is a useful tool for detecting substance use, planning treatment, and monitoring treatment effectiveness for patients with, or at risk for, addiction [24]. In this clinical setting, IVD toxicology immunoassays are often used for initial and ongoing patient assessment due to their fast result turnaround time compared to MS methods. Additionally, MS-based LDTs can be used to

confirm abstinence from certain substances (e.g., ethanol, opioids) or detect relapse, as they can detect even small concentrations of compounds that may be below the cutoff threshold of an immunoassay [25]. MS LDTs can also be used to confirm positive screening results and detect recreational use of substances not detected by screening tests, such as designer drugs like synthetic cannabinoids [26] or herbal supplements containing mitragynine (i.e., kratom) [27]. Non-FDA-cleared immunoassay kits are also available for many emerging drugs of abuse [28,29], and can be used to streamline the LDT development and validation process for laboratories, albeit a longer development process than MS-based versions due to challenges associated with producing and evaluating diagnostic antibodies.

MS-based LDTs are often used in clinical settings to assess compliance with medication-assisted treatment (MAT) regimens, such as methadone or buprenorphine, which can be used to treat opioid substance use disorders. The quantitative evaluation of the parent compound to metabolite ratio can provide further insight into whether the medication is taken as prescribed. For example, a compliant patient on a buprenorphine regimen will have a relatively low level of buprenorphine compared to its metabolite, norbuprenorphine. On the other hand, a patient attempting to adulterate their urine specimen to feign compliance will have a very high buprenorphine level relative to the norbuprenorphine metabolite. Generally, a ratio of the norbuprenorphine to buprenorphine concentration that is < 0.02 is considered indicative of spiking the urine sample with the medication rather than taking it [30]. IVD immunoassays are unable to distinguish the parent drugs from their metabolites and therefore cannot provide this additional level of insight for monitoring adherence to the treatment regimen and the detection of possible drug diversion. Toxicology LDTs improve patient care by enhancing the drug testing used to support the evaluation, diagnosis, treatment, and recovery of patients with substance use disorders.

2.3. Routine care

MS-based LDTs can help prevent toxic drug interactions by identifying and quantifying the presence of substances that may alter the course of patient care but evade detection by standard drug screens. They have also been used in the routine care setting to enhance patient safety by evaluating substance exposures prior to medical procedures. For example, patients may be tested to confirm abstinence from tobacco products prior to an elective surgical procedure since nicotine has been recognized as a risk factor for poor wound healing in postoperative outcomes studies [31]. These tests can detect and quantify the major metabolites of nicotine (cotinine, trans-3′-hydroxycotinine, and nornicotine), which can differentiate between active and passive nicotine exposure based on the measured concentrations [32–34]. Additionally, they can include the quantitation of a tobacco-specific alkaloid, such as anabasine or anatabine, to distinguish nicotine replacement therapy from tobacco use [33,35]. This additional layer of information allows physicians to make more informed clinical decisions about nicotine exposure and facilitates more accurate assessment of patient risk.

Toxicology LDTs can be used to evaluate unexplained symptoms, unexpected responses to treatment, and possible mental disorders by determining if substance use or withdrawal may be contributing to psychiatric symptoms. They can also assist in the diagnostic workup of asymptomatic patients or those with symptoms similar to other disease states. For example, LDTs are often used to detect abnormal heavy metal concentrations (e.g., lead, arsenic, cobalt) due to environmental or occupational exposure, or even exposure via joint replacement implants. An accurate test methodology is essential for quantifying the metal ion level and providing speciation since the chemical form of the metal dictates its degree of toxicity [36–38]. Although IVD tests are available for some metals, labs may choose to use an LDT instead if it can provide additional information that positively impacts clinical decision-making (e.g., speciation) [39] or if the quality of results generated by the FDA-cleared test is in question (e.g., interferences, accuracy) [40].

Toxicology LDTs are particularly useful for confirming drug screening results that may be used to inform other treatment or social decisions, such as postnatal drug screening results obtained on either the mother or the neonate, as they can have severe consequences. False-positive immunoassay results could lead to child protective services unjustly removing a child from their parents, and false-negative results could expose the child to an unsafe environment. Additionally, LDTs offer the advantage of testing non-standard biological matrices such as hair, oral fluid, and meconium, which can provide valuable information about the timing of substance exposure [41]. For example, in neonatal toxicology umbilical cord tissue and meconium drug testing allows for the detection of in utero substance exposure during pregnancy to guide effective management of the newborn [42]. However, many of these alternative specimen types contain very low concentrations of drug(s), drug metabolites, or toxins and require the increased analytical sensitivity and specificity that toxicology LDTs offer. Despite their established clinical utility, FDA-cleared IVDs do not list these specimen types as acceptable. Given the wide variability in specimen type and quality, the considerable resources required, and the current regulatory climate, significant challenges exist for IVD assay manufacturers to expand their list of FDA-cleared specimen types; thus requiring toxicology LDTs to bridge the gap.

2.4. Emergency toxicology

Toxicology testing may be used in the emergency department to investigate whether a drug overdose or toxin is causing life-threatening symptoms, altered consciousness, or abnormal behavior. Treatment of a poisoned patient typically involves supportive care, such as limiting absorption (e.g., activated charcoal) or enhancing excretion (e.g., hemodialysis) of the substance. Rapid identification of a specific toxic syndrome (i.e., toxidrome) or toxic agent can be invaluable for cases in which a specific treatment or antidote is available, such as naloxone for an opioid overdose or digoxin-specific antibody fragments for digoxin overdose. Additionally, drug testing can be used to validate patient-reported drug history, monitor the effectiveness of treatment, and identify potential drug interactions with anesthesia or other medications given to the patient during their care.

Immunoassay is the dominant testing methodology used for managing patients due to the rapid turnaround time required for drug test results. However, there are limitations to the number of compounds that a standard immunoassay workup can detect. Immunoassay-based LDTs can be helpful in expanding a standard drug testing workup to include emerging drugs of abuse, but the availability of the test kits may still lag behind the drug abuse trend. The development and marketing of non-FDA-cleared fentanyl immunoassay kits serves as an example of how LDTs can act as a stopgap while FDA clearance is pursued. MS-based LDTs, both targeted and untargeted, have also demonstrated promise in this setting because they offer a more comprehensive assessment for compounds of interest — natural toxins, pesticides, novel psychoactive substances, and emerging drugs of abuse — that would otherwise be overlooked with traditional IVD immunoassay screening methods and for which immunoassays are unlikely to ever be available [43]. Despite their potential, providing STAT MS-based test results for use in initial management of poisoning or overdose cases remains a challenge. Opportunities to reduce cost constraints and improve testing workflows, such as automation of mass spectrometers or off-site expert data review, offer potential solutions to this challenge and could pave the way for more widespread use of MS-based testing in emergency toxicology.

Most MS-based toxicology testing services cannot currently meet the turnaround time needed for initial patient management in the emergency department; however, the data generated can still have a positive impact on patient care. In complex emergency medicine cases, such as severe or unexplained toxicity, patients are often admitted to an intensive care unit for continued management. Having timely MS drug testing

results available during their hospital stay may help detect unsuspected drugs or lead to changes in patient management. Additionally, pediatric toxic exposure cases are almost exclusively managed using MS-based testing since they typically involve prescription medications or household toxins that cannot be detected by an IVD solution. MS toxicology LDTs can also provide public health benefits by serving as a powerful tool for community surveillance or toxicovigilance.

3. The evolving landscape of LDTs in clinical toxicology

Many LDTs could, in theory, be offered as commercial IVD assays. However, several challenges have driven the continued use of LDTs in clinical toxicology. These include frequent changes in recreational drug availability, the introduction of novel illicit and pharmaceutical drugs, and the evolution of pharmaceutical prescription trends. This dynamic nature of the toxicology landscape necessitates easily customizable panels to account for regional differences in drug abuse trends [44] and prescribing practices [45]. IVD manufacturers are unlikely to offer extensively customized panels due to the time-consuming process of assay design, development, and FDA clearance. Additionally, commercial IVDs are relatively static compared to LDTs and often lag behind quickly evolving drug utilization trends. Nevertheless, large strides have been made in the past decade by the IVD industry to reduce the gaps in clinical care requiring the use of toxicology LDTs.

Although MS LDTs are routinely used in clinical toxicology laboratories, the technical expertise and resources required to develop, implement, and manage them can be prohibitive for many healthcare institutions [46,47]. To make MS testing more accessible, IVD manufacturers have made significant advancements in automation to simplify sample preparation [48–51], increase testing throughput [52], and streamline complex data analysis [53], which will reduce the burden on clinical laboratories performing these LDTs [54,55]. The FDA approval of the first fully automated MS-based analyzer in 2018 was a testament to these efforts [56,57]. Other manufacturers are likely to follow with automated analyzers of their own. Additionally, the IVD industry is shifting the market with nearly every major MS manufacturer now offering IVD versions of their analyzers and software; previously, only Research Use Only (RUO) versions were available that were not intended for clinical diagnostic use but were still routinely used for LDTs.

The launch of commercial testing kit development initiatives for a variety of methodologies has propelled clinical toxicology testing forward over the past decade and shaped the LDT landscape. These kit-based testing solutions utilize a building block system approach, often offering testing parameters, calibrators, quality controls, and reagents. The speed at which these test kits are being designed, developed, and marketed is improving to make them available in a more clinically relevant timeframe. Many are non-FDA-cleared kits intended to streamline the LDT validation process for clinical labs and facilitate a rapid response to shifting drug targets. Some are eventually submitted for FDA clearance by the manufacturer, while others remain as LDTs. The timely creation of these kits, vendor application notes for instrument-specific protocols, and extensive drug/toxin libraries [58] provide clinical laboratories with more support than ever before.

These advancements are promising steps toward addressing the diagnostic testing gap between patient needs and available IVD toxicology tests on a global scale. Making the implementation of high-quality LDTs more manageable for all clinical laboratories, regardless of size or type, will contribute to elevating the standard of patient care in this domain, as will increasing the number of FDA-cleared tests that better meet clinical needs. The landscape of LDTs continues to evolve, but they remain an essential tool in the clinical laboratory toolbox and a key driver of diagnostic innovation.

4. Conclusion

IVDs are essential for clinical toxicology testing, yet they cannot meet all clinical needs at present. LDTs are a crucial part of toxicology testing, enabling us to provide the best care to patients and driving diagnostic innovation. Despite changes in the LDT landscape due to initiatives in the IVD industry and potential legislation reform, LDTs will likely remain an important part of clinical toxicology for the foreseeable future.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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