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CLINICAL LABORATORY TECHNIQUES FOR DETECTING DRUGS OF ABUSE: FEASIBILITY IN DEVELOPING NATIONS

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ABSTRACT

Drugs of abuse are prescription, over-the-counter, or other forms of drugs that are often used for purposes other than those for which they are meant to be used, or in excessive amounts. The abuse of illicit drugs (for example cocaine, heroin, and codeine) poses a serious threat to public health, not to mention a great challenge to the health care system. It is important to review the techniques used in detecting these drugs of abuse to stay ahead of this growing global drug problem. This review aims to give an overview of known clinical laboratory techniques used in detecting some common drugs of abuse that could serve as an integral part of determining the presence of these drugs in bodily fluids and samples. Relevant literature was reviewed in various search engines (Google Scholar, PubMed, ScienceDirect, Bing). Findings showed that some of the current clinical laboratory techniques for detecting and quantifying drugs of abuse include Microcrystalline tests, thin-layer chromatography, colourimetric tests, immunoassays, urine dipstick tests, and ultraviolet spectroscopy. Apart from these known techniques, new and emerging techniques are being validated to serve as additions to these already existing techniques and be used in clinical settings. In conclusion, the burden of drug abuse is on the rise and becoming a public health concern. There should be an ever-increasing interest in developing new analytical methodologies not only to detect but also to quantify drugs of abuse which may be applied in a plethora of areas including clinical settings.Some of the outlined techniques are highly priced and for cost ineffectiveness, they may be difficult to afford and sustain in resource-restraint settings like developing nations. Hence, grants and laboratory infrastructural support may be needed from international donor agencies.

INTRODUCTION

There is a growing interest in creating novel analytical techniques that may be used in a variety of settings to not only identify but also quantify drugs of abuse (DOA). The intended use and desired outcome of the analytical protocol, such as for screening, as is frequently the case with DOA for determining Driving Under the Influence of Drugs (DUID), or for quantification and identification, where analytical equipment like Gas Chromatography-Mass Spectrometry is necessary for forensic toxicology, dictate

the choice of analytical protocols (Bosker et al., 2009). Abuse of illegal drugs is extremely challenging for the healthcare system and constitutes a severe threat to public health. Drug misuse and overdose deaths have surged dramatically over the past few years, approaching epidemic proportions. Since 2008, drug overdose deaths have exceeded those brought on by car collisions and gunfire as the top cause of injury fatalities. 52,404 unintentional overdose deaths occurred in 2015, an 11.4% rise from 2014, according to the Centres for Disease Control and Prevention (CDC), with more than 60% (33,091) of these deaths ascribed to opioids. For the first time, deaths from heroin and non-methadone synthetic opioids, such as illegally obtained fentanyl and its analogues, outnumbered deaths from prescription opioids (Rudd et al., 2016). Since 2007, there has been a dramatic increase in heroin abuse, with 435,000 users in 2014 compared to 161,000 users in 2007. Between 2007 and 2014, there was a 248% increase in heroin overdose deaths. Because heroin is becoming more widely available, some people who abuse prescription drugs switch to heroin as a less expensive option (Frank and Pollack, 2017). Drug misuse is a growing problem in Nigeria and is now a public health issue. Nigeria, the most populous nation in Africa, has earned a reputation as a hub for drug use and trafficking, particularly among young people (Ojebuyi and Salawu, 2015). The aim of this review therefore is to provide an overview of clinical laboratory techniques used in detecting and quantifying drugs of abuse.

Classification of some common Drugs of Abuse

Narcotics: These include Heroin, Morphine, Methadone, Pethidine, Codeine, Demerol, Oxycontin etc.

Cerebral Stimulants: These include cocaine, amphetamine and methyl-amphetamine, phenmetrazine, and some anti-depressants, e.g., nortriptyline and imipramine.

Sedatives: These are barbiturates, glutethimide, and methaqualone, etc.

Hallucinogen: Examples include Cannabis and Lysergic acid diethylamide (LSD) (Beckett and Rowland, 2005).

Biological Specimens used for Analysis of drugs of Abuse

Saliva

Compared to urine, saliva is one of the more interesting biological specimens for identifying recent drug use of psychotropic substances (Vandenes et al., 2012). It is regarded as one of the main justifications in favour of its use by the police to detect narcotics used by drivers of vehicles engaged in traffic accidents in their vehicles, at work, or on the roadside(Bosker et al., 2009). Amphetamine, 3,4-methylenedioxy-methamphetamine (MDMA), cocaine, opiates (heroin and codeine), cannabis, and benzodiazepines are among the substances that can be detected in human saliva (Frank and Pollack, 2017; Ojebuyi and Salawu, 2015). The key advantages of using saliva samples for psychotropic drug screening include their noninvasiveness, convenience of collection, handling that doesn't interfere with the subject's intimacy and the difficulty of adulteration. In actuality, only a visual examination by qualified personnel is necessary (Huestis et al., 2011).

Urine

The most frequent tests utilised by medical professionals are urine drug testing. These examinations for traffic control were adopted by numerous nations, and many positions today demand a screening to apply. Numerous studies have utilised urine as a biological matrix for drug analysis (Mercieca et al., 2018).The detection of illicit drugs in the urine reveals information about past or present use. The implementation of urine screening assays, however, is more challenging due to the significant limitations placed on sampling. The reasons urine tests are more popular are that they are non-

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invasive, quick, provide a big sample volume, and can subjectively detect a variety of drug compounds (Lee et al., 2013).

Blood

Comparing blood samples to saliva or even urine, blood samples are the most difficult to get practically. It must be done in a lab by trained medical professionals. Although it takes time, this procedure occasionally determines whether a test is accurate enough to be positive or negative. Blood tests can be used to measure the levels of specific drugs of abuse and their metabolites, but because they are an invasive procedure, they are less frequently used (Barbosa et al., 2006). Blood, as opposed to urine, has the benefit of never being tainted, and a confirmed relationship between the amount absorbed and the blood level and, consequently, effects on the central nervous system (dose/concentration relationship and dose/effect) has been established (Wang et al., 2010). In comparison to urine, the blood has a smaller detection window and lower concentrations. Blood is the most significant and useful specimen for drug analysis in both forensic science and clinical chemistry. Blood samples have been used frequently to detect cocaine, amphetamines, and opiates while analyzing drugs of abuse (Kiss et al., 2010).

Meconium

For the past 20 years, the preferred specimen has been meconium since it has various advantages for identifying prenatal drug use. The meconium acts as a reservoir for exogenous substances, such as medications and metabolites, and accumulates substances from the 12th gestational week until birth. Fetal biliary excretion of drugs or consumption of drug-contaminated amniotic fluid results in the incorporation of drugs into the meconium. Neonatal hair and urine studies show more recent exposure, whereas meconium analysis is thought to enable the detection of maternal drug use during the second and third trimesters of pregnancy. Meconium is typically passed within the first 1 to 3 days of life, however, in premature infants, this process may be postponed. Drug isolation is challenging due to the complex composition of mucopolysaccharides, water, bile salts, bile acids, epithelial cells, and other lipids (Gray et al., 2009).

Placenta

During the first trimester of pregnancy, the placenta may be used as an alternative specimen to urine for testing illegal drugs. The advantage of placenta tissue over more traditional matrices like blood and urine is that collection is practically noninvasive and manageable. Placenta tissue is obtained as wasted material in the event of pregnancy termination. Joya et al. (2010) reported the development and validation of a method for the quantification of drugs of abuse in human placenta tissue, including cocaine, benzoylecgonine, morphine, nicotine, and cotinine.

Brain Tissue

In toxicology laboratories, postmortem drug screening is frequently done in cases when an overdose is thought to be the cause of death. Due to its temporal relationship with a drug's effect on a person and his or her condition at the time of death, blood is the most frequently employed specimen for examination. In addition to blood analysis, other specimens such as vitreous humour, saliva, and urine are frequently examined, especially when blood extraction is problematic for one reason or another. There is strong evidence showing that medicines and their metabolites mostly disperse in lipid-rich tissues like the brain and adipose tissues. Brain tissue offers good information on a person's condition, potential physical changes, and behaviour at the moment of death (Popa et al., 2009). When a postmortem inquiry is conducted to ascertain the likely cause and method of death, the study of brain tissue specimens may offer useful information as the type of drug supplied (Shakleya and Huestis, 2009).

Adipose Tissue

Adipose tissue can be used to detect absorbed chemicals in living organisms, provided that

the chemicals are highly lipophilic and bind to them. Since chemicals that are absorbed by the body tend to persist in adipose tissue due to their lipophilicity, higher storage of chemicals in adipose tissue in mammals suggests a longer exposure of the tissue to the chemicals even after the cessation of external exposure. The adipose tissue/blood partition coefficient is connected with the degree of chemical buildup in adipose tissue. For instance, THC is quickly absorbed by tissues, including adipose tissue, and is highly soluble in lipids (Colucci et al., 2010).

Sweat

Sweat has attracted attention in recent years as an alternative matrix in forensic toxicology as it can be sampled easily and non-invasively. Sweat can be collected with patches over a long period, thereby permitting drug monitoring over a longer period than urine. Concheiro et al. (2011) developed a method for the quantification of cocaine, benzoylecgonine, morphine, codeine, heroin, 6-acetylcholine, and cotinine in sweat specimens.

Breath

Berchtold et al. (2011) tried to detect such narcotics as morphine, fentanyl, nor fentanyl, naloxone, cocaine, hydroxy butyrolactone, and nicotine from breath. They challenged the diagnosis by online breath analysis using Mass spectrometry, because of the low concentrations of the pertinent compounds in breath.

Nail Clippings

Nail clippings were used as analytical specimens for the detection and quantitation of illicit drugs. Kim *et al*. developed a GC-MS method for the simultaneous qualification and quantification of amphetamine, methamphetamine, MDA, MDMA, ketamine, and nor ketamine in nail clippings (Kim et al., 2010).

Clinical Laboratory Techniques for Detecting and Quantifying Drugs of Abuse

Microcrystalline Tests

When a particular reagent is used in these chemical tests, distinct microcrystals of a given analyte develop. Using a typical light microscope, the novel crystal formation is compared to a reference standard/control. Based on form, size, colour, and spatial arrangement, microcrystals are contrasted. Cocaine, heroin, methadone, GHB (gamma hydroxybutyrate), ketamine, phencyclidine, amphetamines, and methamphetamine are just a few of the drugs that are frequently misused (Elie and Elie, 2009). The advantage of microcrystalline testing is its affordable price. Reagents are needed in trace concentrations. Although the instrumentation is straightforward, this method cannot quantify the amount of a chemical that is present. Unfortunately, the method results in the sample being destroyed, which may not be optimal for those bringing the samples for identification.

Microcrystalline test for cocaine

This uses 5% gold chloride or 5% platinic chloride as a reagent and requires the sample to be dissolved in 10% acetic acid or HCl. The characteristic microcrystals expected to be seen are serrated needles with gold chloride and thin needlelike branched skeletons with platinic chloride (Bell and Hanes, 2007).

Microcrystalline test for Heroin

Diamorphine is dissolved in 10% hydrochloric acid and microcrystalline is tested using 1% mercuric chloride or 1% mercuric iodide both resulting in dendritic crystal formations (Clarke, 1975).

Thin Layer Chromatography

Through the use of a planar stationary phase and a liquid mobile phase, thin-layer chromatography (TLC) uses capillary action to separate samples. The retention time of the analyte depends on whether it has been

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adsorbed to the stationary phase or is in the mobile phase and on how long it has been there. Depending on the component's size and affinity for the mobile phase, components of the sample move at varying speeds (Kanai et al., 2008). As a result, a plate of dots (separated mixture elements) that have traveled different distances on the stationary phase is produced. Barbiturates, benzodiazepines, GHB, heroin, morphine, opium, oxycodone, and other opiates, cocaine, MDMA (methylenedioxymethamphetamine or Ecstasy), ketamine, LSD, marijuana, mescaline, synthetic cannabinoids, and cathinone (often referred to as "bath salts") can all be detected using TLC. It might be challenging to separate and distinguish novel psychoactive compounds using TLC (Cargill and Kammrath, 2014). TLC struggles to effectively separate complicated combinations. The range of micro-nanograms is sensitivity. Measured retention factors can be used to make a preliminary identification of a chemical but are not specific to a single component, and specificity can range from intermediate to high depending on the mixture (Cargill and Kammrath, 2014).

Colourimetric Tests

Presumptive testing based on chemical interactions between analytes and indicators is provided by colourimetric tests. Cobalt thiocyanate, Mandelin, Marquis, para-dimethylaminobenzaldehyde, ferric chloride, Froehde, Mecke, Zwikker, and Simon's (nitroprusside) are only a few examples of the numerous indicator tests that could be used (O'Neal et al., 2000). Depending on the analyte being analysed, the indicator and analyte interact chemically and produce a response that results in a specific colour staining. Then, spots are visually compared to reference charts; the Munsellcolour charts are the current standard. By employing a straightforward smartphone app to accurately identify colours and accompanying software to match the results in a searchable database, it is possible to avoid the human eye and its subjectivity (Elkins et al., 2017). For the majority of commonly abused substances, such as cocaine, various prescription opioids, amphetamines, LSD (lysergic acid diethylamide), cathinone (bath salts), heroin, and fentanyl, colourimetric tests are available. Other novel psychoactive chemicals might exist, but no accompanying colourimetric assays exist for them (yet). Details on the analytes that can be employed with each particular specified test will be provided. Sadly, the test also destroys the supplied sample. Nevertheless, colour tests only need a small sample; as long as the object can be seen, it can be assessed. Depending on the analyte and the spot test used, colourimetric assays can be quite sensitive, with limits of detection in the microgram range (O'Neal et al., 2000).

Immunoassay

An antibody that is specific for the drug or drug class of interest (antigen) is bound in an immunoassay, along with a label that will be a component of the antibody-antigen complex and can be detected in some way (such as fluorescence). In a typical immune response, antibodies in biological tissue bind to antigens toneutralise or remove them. This is the basis for antigen-antibody binding. Because these techniques were initially intended for the examination of biological materials (mainly metabolites in urine), they are rarely used in drug analysis. So, traditionally, immunoassay does not provide a determination of the type or amount of a drug before its intake or administration but does provide vital patient information for clinicians. However, additional biochemical assays for the identification of an analyte in a liquid sample can be carried out using ELISA. Using immunoassay technology, different opioids and cocaine can be quickly detected. Due to similarities in drug structures or metabolites, immunoassays frequently produce false positive results and have issues with specificity. Given that antibody-antigen interactions take place at the molecular level, sensitivity is fairly good with detection in the microgram range (Harper et al., 2017).

Urine Dipstick Tests

This technique has lately attracted interest as a reasonably priced, simple-to-use fentanyl presumptive test (Lysyshyn et al., 2018). A small amount of the drug is dissolved in water, and if the amount of fentanyl it contains is greater than the cut-off levels, an indicator will show up on the test strip. The procedure uses a chromatographic immunoassay, and when the right analyte is present, a strip on the indicator changes colour. The only substance for which this kind of drug screening has reportedly been employed to date is fentanyl (Sorak., 2012).

Ultraviolet Spectroscopy

This technique is based on the absorption of ultraviolet (UV) wavelength light radiation. The energy levels of the electrons within a molecule can be raised from their ground state to higher energy levels by light in this range. Each transition to a higher energy level needs a specific amount of energy, which light with a specific wavelength can provide. The electrical structure of the entire molecule will decide which wavelengths are absorbed versus which pass through a sample, therefore using a specific wavelength of light, a distinctive UV absorption spectrum can be created. To establish a characteristic spectrum, UV-vis (ultraviolet-visible) spectrophotometers assess the intensity of light passing through a sample, compare it to the intensity of light before it passes through the sample, and record this data. Drugs with comparable structural qualities could have the same UV spectra. UV-vis has been utilised to specifically identify six separate compounds. Concentrations of MDMA, ketamine hydrochloride, cocaine hydrochloride, diazepam, phenobarbital, and barbital in the microgram range have also been identified. Although there is no literature on confirmed usage for a wide range of illegal drugs, other chemicals may be discernible. For increased selectivity and specificity, UV can be used in conjunction with chromatographic methods. The detection of many substances in a combination is not appropriate. To prevent saturated spectra from being produced by the procedure, samples must be diluted (Bunaciu et al., 2010).

Mass Spectrometry

The current gold standard in laboratory drug analysis is mass spectrometry, which precisely determines the molecular mass of ions as defined by their mass-to-charge ratio (m/z) (Cody et al., 2005). Mass spectrometry typically needs separation, ionization, and detection. Gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE) are all methods for separation. Different ionization techniques exist. Electron ionisation (EI), atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI), matrix-assisted laser desorption ionization (MALDI), atmospheric pressure photoionization (APPI), fast atom bombardment (FAB), and more recently direct analysis in real-time (DART) are the techniques most frequently used in the analysis of illicit substances. Using MS in conjunction with a separation (chromatographic) method, almost any substance can be detected. Current mass spectrometers are sensitive enough to identify analytes at concentrations as low as attomolar (1018) (Forsgard et al., 2010). Due to the mass-charge filter analyzer's ability to decrease background interference and create a crisper result and analyte fingerprint, MS offers higher sensitivity than some other analytical techniques. Due to its distinctive fragmentation patterns, high resolution, and exceptional filtering capabilities—especially when used in tandem or higher-order mass spectrometry—it exhibits good specificity (Manimala et al., 2016). The creation of gas phase ions of the molecule, primarily through electron ionisation, is the first step in the mass spectrometric study of substances. This molecular ion splits into pieces. Fragmentation occurs in turn for each major product ion formed from the molecular ion, and so forth. The mass spectrometer separates the ions based on their mass-to-charge ratio, and they are detected proportionally to their

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abundance. This results in the creation of the molecule's mass spectrum. The outcome is shown as a plot of ion abundance against the mass-to-charge ratio. Ions reveal details about the makeup and structure of their precursor molecule. The molecular ion, if present, gives the molecular mass of the molecule by appearing in the spectrum of a pure compound at the highest value of m/z (followed by ions carrying heavier isotopes) (Sisco et al., 2017).

Nuclear Magnetic Resonance Spectroscopy

One of the most effective methods and useful instruments for obtaining comprehensive details regarding the composition, behaviour, and interactions of both organic and inorganic pharmaceuticals is nuclear magnetic resonance (NMR) spectroscopy. This method has also shown promise for both qualitative and quantitative analysis of absorbing species like benzodiazepines. In fact, according to a study, Metizolam was consistently found in amounts lower than 11 ng/mL in hydrolyzed urine during the 46 hours. As a result, analytes present in the sample, such as urine, can be quickly detected and identified because of the extremely high information richness of the resulting NMR spectra (Kintz et al., 2017). NMR spectroscopy's nondestructive nature, which enables future reanalysis of the sample using alternative techniques, is another advantage (Nyitrainé et al., 2022).

CONCLUSION

The abuse of illicit drugs poses a serious threat to public health, not to mention a great challenge to the healthcare system. Some of the drugs often abused include cocaine, heroin, cannabis, amphetamine, and codeine. There is a need to always update the laboratory techniques used in the detection and quantification of these drugs to combat this serious threat of drug abuse. Some of the available clinical laboratory techniques for detecting these drugs include Urine dipstick test, immunoassay, microcrystalline test, ultraviolet spectroscopy, and thin layer chromatography. Some of the outlined techniques are highly priced and for cost ineffectiveness, they may be difficult to afford and sustain in resource-restraint settings like developing nations. Hence, grants and laboratory infrastructural supportmay be needed from international donor agencies.

RECOMMENDATION

Drug abuse testing plays a vital role in various settings, including healthcare, workplace safety, forensic investigations, and addiction treatment. We recommend that reliable testing methods should be made available to identify the presence of drugs and ensure appropriate intervention and support for individuals affected by substance abuse. It would be important to emphasize that resources in developing nations would be inadequate to acquire and sustain some of these outlined technologies, hence there is a need for support from donor agencies focusing on healthcare diagnostic systems enhancement.

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