Research Article

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Point of Care Immunoassay Drugs of Abuse Screening in a Post Mortem Population

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ABSTRACT

To test the utility of point-of-care immunoassay testing of drugs of abuse in a post-mortem population, we compared immunoassay near-body drugs of abuse results with liquid/gas chromatography-mass spectrometry confirmatory testing. While immunoassay results are subject to false positives from decompositional amines and prescription medications, negative screening tests can produce significant cost-savings and allow for timelier autopsy reports where confirmatory testing is unnecessary. This would also help ease the burden on reference and/or State laboratories and save approximately 500,000 euros per annum across the health system.

Keywords

Immunoassay, Post-mortem, Near-body drugs of abuse.

Abbreviations

SUH: Sligo University Hospital, DOA: Drugs of abuse, POC: Point of care, LC/GC-MS: Liquid Chromatography/Gas Chromatography-Mass Spectrometry, AMP: Amphetamine, MAMP: Methamphetamine, MDMA: Methylenedioxymethamphetamine, OXAZ: Oxazepam, CLNZ: Clonazepam, TCA: Tricyclic Antidepressants, BENZO: Benzodiazepine, ZOLP: Zolpidem, PGB: Pregabalin, BZG: Benzoylecgonine, THC: Tetrahydrocannabinol, EtOH: Ethanol, OPIAT|: Opiate, KET: Ketamine, XYL: Xylazine, TRAM: Tramadol, ACE: Acetaminophen, FEN: Fentanyl, MDONE: Methadone, OXY: Oxycodone.

Introduction

Toxicology results in post mortem examinations can be critical to cause of death and have potential legal implications, for example, in road traffic accidents [1]. While liquid/gas chromatography mass spectrometry (LC/GC-MS) is the gold standard for drugs of abuse (DOA) testing [2-6], point-of-care (POC) immunoassay testing has many potential advantages such as speed, ease of use and cost efficiency [7-10]. Toxicological analysis can be fraught with delay, partly due to overwhelming number of samples submitted to large reference toxicology laboratories. Locally, for example, there has been a significant increase in toxicology samples being sent to the State Laboratory from circa 5000 annually to 8700 annually and forecasted to reach 9000-10,000 in the coming years. Turnaround time for results can be up to 9 months. Local use of POC immunoassay testing at the time of post-mortem examination

would allow for results in 20 minutes [11,12], and potentially help ease the burden on reference laboratories, offer significant cost savings and allow for more expedient autopsy reporting [7]. Comparison of results from immunoassay DOA testing with LC/GC-MS confirmatory results to evaluate the potential utility of immunoassay POC testing in a post-mortem population were performed.

Whole blood samples were taken from femoral vessels of decedents. Blood samples were processed and qualitatively recorded using near-body POC immunoassay (Randox Evidence MultiSTAT) and confirmed with mass spectrometry methods (LC/GC-MS) as utilised by the State Toxicology Laboratory. Methamphetamines (MAMP), methylenedioxymethamphetamine (MDMA), amphetamine (AMP), tricyclic antidepressants (TCA), opiates (OPIAT), oxazepam (OXAZ), clonazepam (CLNZ), zolpidem (ZOLP), tetrahydrocannabinol (THC), benzoylecgonine (BZG), pregabalin (PGB), oxycodone (OXY), methadone (MDONE), tramadol (TRAM), acetaminophen (ACE), fentanyl (FEN), ketamine (KET), xylazine (XYL) and ethanol (EtOH) were the drugs of abuse examined in this preliminary study.

Materials and Methods

Following ethics approval from Sligo University Hospital Research and Education Ethics Committee, this was a prospective, observational study examining a sample population from the Pathology Service Mortuary. Whole blood samples were taken from femoral vessels of decedents. Blood samples were processed and qualitatively recorded using near-body POC immunoassay (Randox Evidence MultiSTAT) and confirmed with mass spectrometry methods (LC/GC-MS) as utilised by the State Toxicology Laboratory. Methamphetamines (MAMP), methylenedioxymethamphetamine (MDMA), amphetamine (AMP), tricyclic antidepressants (TCA), opiates (OPIAT), oxazepam (OXAZ), clonazepam (CLNZ), zolpidem (ZOLP), tetrahydrocannabinol (THC), benzoylecgonine (BZG), pregabalin (PGB), oxycodone (OXY), methadone (MDONE), tramadol (TRAM), acetaminophen (ACE), fentanyl (FEN), ketamine (KET), xylazine (XYL) and ethanol (EtOH) were the drugs of abuse examined. Positive predictive value (PPV), negative predictive value (NPV), and diagnostic sensitivity and specificity were calculated with standard formulae. Cohen's kappa determined inter-method agreement and reliability.

Randox Immunoassay Screening System

The Randox immunoassay-screening test is an automated system that is able to use whole blood or urine as sample matrix. This system requires the use of the automated Evidence MultiSTAT, a compact, laboratory based, semi-automated benchtop platform that utilises the Biochip Array Technology. This analyser accommodates simultaneous detection of multiple drug metabolites from a single sample, with the ability to consolidate a number of immunoassay tests. The system is kit based and provides for all the necessary components such as the chips, chemicals, calibrators and developing agents. It consists of nine 9 x 9 mm biochips in wells on a cassette.

Confirmatory Testing

Liquid or gas chromatography coupled with mass spectrometry are considered gold-standard analytical techniques. The general principal of chromatography is the separation of a mixture of substances or analyte into its individual constituents. These individual constituent molecules are then detected and identified using mass spectrometer.

Results

One hundred and four decedents, 96 male and 8 female, ranging in age from 19 to 107 (m=65), were tested using the POC DOA immunoassay analyser. Thirty-four were negative for all analytes tested. Four samples were rejected due to severe post-mortem decomposition. Seventy had LCMS results reported for comparison or were LCMS-negative. Of the 66 samples positive for one or more analyte, 35 were positive for AMP, MAMP, and MDMA, 17 for OXAZ and CLNZ, 18 for OPIAT, OXY and MDONE, 5 for ZOLP, 5 for PGB, 7 for THC, 6 for BZG, 7 for ACE, 1 for KET, 1 for FEN and 28 for EtOH for a total of 135 positive blood results. Of these, 48 were confirmed (36%) with positive results from the State Laboratory LCMS at the time of this report: AMP 0/25 confirmed, MAMP 0/5 confirmed, MDMA 0/5, OPIAT 5/13, MDONE 1/1, OXY 0/4, TRAM 1/2, OXAZ 5/11, CLNZ 3/6, ZOLP 3/5, PGB 2/5, THC 0/7, BZG 5/6, TCA 1/3, ACE 4/7, KET 0/1, FEN 1/1 and EtOH 17/28. XYL was negative. All immunoassay results were above State cutoffs. Nine EtOH positive results from State were below immunoassay cutoffs, but not quantitatively clinically significant (<52mg %) and considered negative. (Figure 1). Statistical analyses showed PPV=0.36, NPV=1.00, sensitivity=100%, specificity=28%; Cohen's kappa= 0.181.

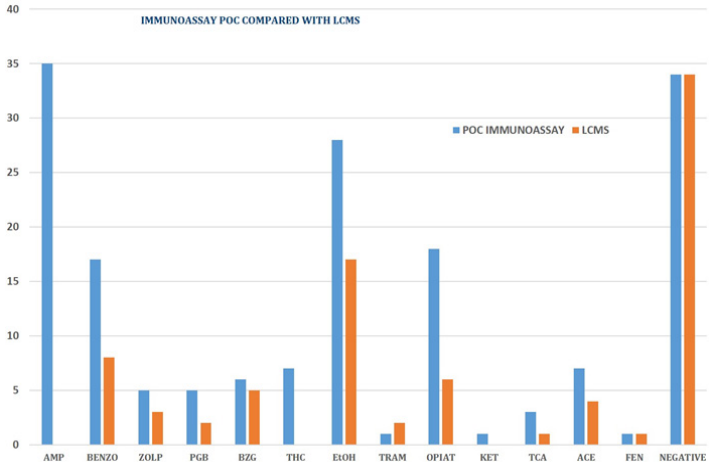

Figure 1: Immunoassay results and LC/GC-MS confirmation.

Discussion

Toxicology results in post mortem examinations can be critical to cause of death and have potential legal implications [1]. While LC/GC-MS is the gold standard for DOA testing [2-6], POC immunoassay testing has many potential advantages such as speed, ease of use and cost efficiency [7-10]. Toxicological analysis can be delayed, partly due to overwhelming number of samples submitted to large reference toxicology laboratories.

Point of care immunoassay testing at the time of post-mortem examination would allow for results in 20 minutes [11,12], and potentially help ease the burden on reference laboratories, offer significant cost savings and much improved turnaround times in autopsy reporting [7].

McLaughlin et al. [12] examined the use of the Randox biochip immunoassay technology for use in a European post mortem population. In this study liver, psoas muscle, femoral blood, vitreous humour and urine from 75 post mortem cases were screened for drugs of abuse. Positive results were confirmed using LC/GC-MS. There was excellent concordance between the immunoassay and gold standard methodology, with correlations between 98 and 100 percent with the various drug groups. There were a few false negative results in certain matrices (for example, cannabinoid and benzodiazepines in vitreous humor). The authors attribute this largely to drug redistribution, drug accumulation and selective membrane permeability. They also mention that in cases of low drug concentrations, especially with respect to opiates and methadone; the immunoassay cut-off levels were higher than the actual blood levels. This resulted in false negative results in those cases. The only false positives detected were for amphetamines. These were detected in two heavily decomposed bodies, which comprised two percent of the study population. This is consistent with what is expected using other methods to detect amphetamines, as the putrefactive amines that are released during decomposition are similar in structure to the amphetamine group of drugs resulting in false positive tests. The authors conclude that the Randox DOA assays can be used in a post mortem population to screen blood, amongst other specimens, for the drugs that the assay is designed to detect. Our comparisons were much poorer than McLaughlin et al.'s with a PPV of 36% and Cohen's kappa showed only slight agreement between immunoassay and LC/GC-MS results. This may have to do with their samples which included liver, psoas muscle, vitreous humour and urine. drug redistribution, drug accumulation and selective membrane permeability.

Immunoassays, even when LC/GC-MS is considered the gold standard for many analytical applications, can still be useful in several scenarios, such as high throughput screening, point-of-care testing, targeted detection of specific analytes, quantitative measurement of biomarkers, longitudinal or routine monitoring and when LC/GC-MS is not available or accessible [2-6]. They require less sample preparation and are cost efficient. Limitations of immunoassays include cross-reactivity, less sensitivity compared with LC/GC-MS and typically require confirmation with LC/GC-MS, [13,14] particularly for cause-of-death and medicolegal implications. Point of care testing can be useful in the context of an autopsy, although it is not typically a primary tool. However, POC testing, especially when negative, can save considerable costs incurred from confirmatory testing. Point of care testing testing can also be useful in screening for infectious diseases, assessment of blood biomarkers, and in field investigations.

Conclusion

Immunoassay results are subject to false positives with many analytes such as AMP and derivatives due to decomposing amines/amino acids in post-mortem samples. Compounds like AMP and THC can show false positives with numerous commonly prescribed medications. While for most analytes, the gold standard of testing for drugs of abuse in post-mortem samples remains LC/GC-MS, immunoassay screening can be cost saving (~7,000 Euro per ~100 post-mortem cases) where negative results do not need further testing. In general, immunoassay POC testing represents a promising tool for forensic pathology and postmortem toxicology. Its ability to provide rapid, cost-effective, and versatile results could significantly improve the efficiency and accuracy of postmortem investigations and reporting. While challenges such as lower sensitivity and the need for confirmatory testing of positive results remain, the potential benefits in terms of speed, accessibility, and applicability to a wide range of biomarkers make immunoassays an important addition to postmortem laboratory practices. Future research and technological improvements may further expand the role of immunoassays in forensic investigations, ultimately contributing to more effective and timely determinations of cause of death.

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References

1. Fatteh, Abdullah. Handbook of Forensic Pathology. Philadelphia, PA: Lippincott Williams and Wilkins. 1973.
2. Robert AE. Liquid Chromatography-Mass Spectrometry: An Introduction. 4th edition. Chichester, United Kingdom: John Wiley and Sons. 2005.
3. Maurer HH. Advances in analytical toxicology: The current role of liquid chromatography-mass spectrometry in drug quantification in blood and oral fluid. Anal Bioanal Chem. 2005; 381: 110-118.
4. Maurer HH. Position of chromatographic techniques in screening for the detection of drugs or poisons in clinical and forensic toxicology and/or doping control. Clin Chem Lab Med. 2004; 41: 1310-1324.
5. Marquet P. Progress of liquid chromatography-mass spectrometry in clinical and forensic toxicology. Therapeutic Drug Monitoring. 2002; 24: 255-276.
6. Karasek FW, Clement RE. Basic Gas Chromatography-Mass Spectrometry: Principles and Techniques 3rd Edition. Amsterdam, The Netherlands: Elsevier Science. 1988.
7. Smith A. Cost-effectiveness of immunoassay POC tests in forensic toxicology: A comparative analysis. Journal of Forensic Toxicology. 2021; 39: 143-151.
8. Sambrook J. Immunoassay-based testing for postmortem diagnostics: Clinical and forensic implications. Journal of Forensic Sciences. 2019; 64: 332-340.
9. Carmichael J. Immunoassays for the detection of infectious agents in postmortem cases: A review of their use and potential. Forensic Science International. 2017; 279: 134-141.

10. Patel M. Immunoassays for forensic toxicology: Advances in point-of-care testing and practical applications. Forensic Medicine. 2020; 44: 536-544.
11. McLaughlin P, Pounder D, Maskell P, et al. Real-time near-body drug screening during autopsy I: use of the Randox biochip drugs of abuse DOA I and DOA II immunoassays. Forensic Toxicol. 2013; 31: 113-118.
12. McLaughlin P, Maskell PD, Pounder D, et al. Use of the Randox Evidence MultiSTAT immunoassay system for near-body drug screening during post-mortem examination in 261 forensic cases. Forensic Sci Int. 2019; 294: 211-215.
13. Fisher K. Limitations and challenges of immunoassay POC testing in postmortem toxicology. Journal of Forensic Pathology. 2016; 12: 184-190.
14. Brahm NC, Yeager LL, Fox MD, et al. Commonly prescribed medications and potential false-positive urine drug screens. Am J Health Syst Pharm. 2010; 67: 1344-1350.