



Amphetamines and Ecstasy (MDMA): A Comprehensive Study Using Siemens and Thermo Fisher Immunoassays Together with LC-MS/MS Method

Ashraf Mina^{*1,2}, Leah McNeice¹, Shanmugam Banukumar¹, Santiago Vazquez¹

¹NSW Health Pathology, Forensic & Analytical Science Service (FASS), Toxicology Unit, Macquarie Hospital, NSW, Australia.

²Affiliated Senior Clinical Lecturer, Faculty of Medicine and Health, Sydney University, NSW, Australia.

***Corresponding author:** Dr. Ashraf Mina, NSW Health Pathology - Forensic & Analytical Science Service PO Box 53, North Ryde Mail Centre, North Ryde NSW 1670

Received Date: December 03, 2021

Published Date: December 15, 2021

Abstract

Drugs of Abuse (DOA) screening methods are regularly used in pathology laboratories to screen and monitor drugs. We conducted two comprehensive separate studies to evaluate amphetamines and ecstasy (MDMA) using Thermo Fisher and Siemens Immunoassay against LC-MS/MS method. These studies aimed to compare the two methods thoroughly using a large number of patients, a total of 34,780 patients, to evaluate the clinical impact on reporting either false positive or false negative results using these methods. We reviewed 547 articles using wider search parameters in PubMed where 68 articles were related to immunoassays. Separate studies were conducted to examine cross-reactivity as mentioned in the Discussion section in this article. This study identified that Thermo Fisher and Siemens immunoassays methods had no false-negative results. Siemens EMIT II Immunoassay gave 1.7 % false-positive results and Thermo Fisher CEDIA immunoassay gave 1.5 % false-positive results.

Keywords: Amphetamines; Ecstasy; MDMA; Immunoassays; Drugs of Abuse

Abbreviations: CEDIA: Cloned Enzyme Donor Immunoassay, DOA: Drugs of Abuse, DRI: Diagnostic Reagents Inc, ELISA: Enzyme-Linked Immunosorbent Assay, EMIT: Enzyme-Multiplied Immunoassay Technique, MDMA: 3,4-methylenedioxymethamphetamine

Introduction

ELISA amphetamine, methamphetamine, and ecstasy (MDMA) kits are extremely effective for the target drugs for which they are intended but cannot be used to reliably identify the tested designer drugs in real cases, as these concentrations greatly exceed those expected to be found in forensic samples [1]. Immunoassays are designed to measure the primary drug for the method. Detection of other new designer drugs or similarly structured drugs would depend on their cross-reactivity [2-5]. Some amphetamine and ecstasy urine immunoassay kits are prone to false-positive results due to the poor specificity of the antibody or interferences [6].

Experimental

We conducted two comprehensive separate studies to evaluate Thermo Fisher and Siemens Immunoassay against LC-MS/MS. These studies aimed to compare the two methods thoroughly using a large number of patients, a total of 34,780 patients, to evaluate the clinical impact on reporting false positive or false negative results using these commonly used screening methods. We reviewed 547 articles using wider search parameters in PubMed where 68 articles were related to immunoassays. No statistics were published regarding that. Separate studies were conducted to examine cross-

reactivity as mentioned in the Discussion section in this article.

Materials and Methods

Immunoassays were evaluated on Beckman-Coulter AU5810 analysers. LC-MS/MS (AB Sciex QTRAP 5500) was used to determine the validity of the outcome. The reagents obtained from Thermo Fisher and their part numbers are CEDIA amphetamines/ecstasy reagents (CDF100040), CEDIA multi-drug optional calibrator (CDF100033), and CEDIA multidrug optional low and high quality controls (100069).

The reagents obtained from Siemens and their part numbers are EMIT II Plus amphetamine (10445421), EMIT II Plus ecstasy

(10445478), EMIT II multidrug calibrator (110445407), 6-AM/ecstasy calibrator Level 2 (10470442), Stands/control low and high-quality control for amphetamines (18003225) and advanced control low and high for ecstasy (18003226). For LC-MS/MS (AB Sciex QTRAP 5500) UTAK validity control 2 (10445225) and UTAK validity control 5 (10445228) were used.

Results

We analysed 17,433 samples using EMIT II plus amphetamines and ecstasy immunoassays against LC-MS/MS. (Table 1). No false-negative results, but 296 patients' results gave false positives using the Siemens EMIT II immunoassay.

Table 1: Siemens Immunoassay and LC-MS/MS Comparison. The total number of patients tested was 17,433.

True Positives	True Negatives
2,113 Patients	15,320 Patients
12.1 %	87.9 %
False Positives	False Negatives
296 Patients	0 Patients
1.70 %	0 %

Then in another study, we analysed 17,347 samples using Thermo Fisher amphetamine/ecstasy combined immunoassay against LC-MS/MS. (Table 2) No false-negative results, but 265

patients' results were false positive using Thermo Fisher CEDIA immunoassay.

Table 2: Thermo Fisher Immunoassay and LC-MS/MS Comparison. The total number of patients tested was 17,347.

True Positives	True Negatives
1,916 Patients	15,431 Patients
11.1 %	88.9 %
False Positives	False Negatives
265 Patients	0 Patients
1.50 %	0 %

Discussion

This study screened a total of 34,780 patients using Thermo Fisher and Siemens immunoassays. In terms of false positives (FP), Thermo Fisher immunoassay performed slightly better (1.5 % FP) than Siemens immunoassay (1.7% FP). Immunoassays are a reliable tool in testing samples for drugs of abuse [7]. A study confirmed the interference of tetracaine in the CEDIA amphetamine/ecstasy immunoassay where 80% of the false positive amphetamine cases were admitted to the emergency department [8]. Also, amphetamines and MDMA immunoassays gave false-positive results in the presence of metoprolol. Therefore analytical interferences with immunoassays and detailed medication history are important for interpretation [9]. Cross-reactivity is a crucial factor in the interpretation of the results [10].

Fenofibrate's interference with DRI immunoassay for MDMA could lead to false-positive results. Fenofibrate is widely prescribed to reduce the amounts of fatty substances such as cholesterol and triglycerides [11-13]. Another study showed that Trazodone, meta-chlorophenylpiperazine (a hallucinogenic drug and trazodone metabolite), and the hallucinogenic trifluoromethylphenylpiperazine cross-react with the EMIT II ecstasy immunoassay in urine and give a false positive [14].

Conclusion

The article identified that Thermo Fisher and Siemens semiquantitative immunoassays methods had no false-negative results. Siemens EMIT II Immunoassay gave 1.7 % false-positive results and Thermo Fisher CEDIA immunoassay gave 1.5 % false-positive results.

Compliance with Ethical Standards

Funding: No funds were received for this article.

Ethical approval: This article does not contain any studies with human participants or animals performed by the author.

Acknowledgement

None.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. M. Nieddu, L. Burrai, E. Baralla, V. Pasciu, M.V. Varoni, et al., (2016) ELISA Detection of 30 New Amphetamine Designer Drugs in Whole Blood, Urine and Oral Fluid using Neogen® "Amphetamine" and "Methamphetamine/MDMA" Kits. *J Anal Toxicol* 40(7): 492-497.
2. L.E. Regester, J.D. Chmiel, J.M. Holler, S.P. Vorce, B. Levine (2015) Determination of designer drug cross-reactivity on five commercial immunoassay screening kits. *J Anal Toxicol* 39(2): 144-1451.
3. O. Beck, L. Rausberg, Y. Al-Saffar, T. Villen, L. Karlsson, et al. (2014) Detectability of new psychoactive substances, 'legal highs', in CEDIA, EMIT, and KIMS immunochemical screening assays for drugs of abuse. *Drug Test Anal* 6(5): 492-499.
4. M. Petrie, K.L. Lynch, S. Ekins, J.S. Chang, R.J. Goetz, et al., (2013) Cross-reactivity studies and predictive modeling of "Bath Salts" and other amphetamine-type stimulants with amphetamine screening immunoassays. *Clin Toxicol (Phila)* 51(2): 83-91.
5. K. Nakanishi, A. Miki, K. Zaitzu, H. Kamata, N. Shima, et al., (2012) Cross-reactivities of various phenethylamine-type designer drugs to immunoassays for amphetamines, with special attention to the evaluation of the one-step urine drug test Instant-View™, and the Emit® assays for use in drug enforcement. *Forensic Sci Int* 217(1-3) (2012) 174-81.
6. S.J. Marin, K. Doyle, A. Chang, M. Concheiro-Guisan, M.A. Huestis, et al., (2016) One Hundred False-Positive Amphetamine Specimens Characterized by Liquid Chromatography Time-of-Flight Mass Spectrometry. *J Anal Toxicol* 40(1): 37-42.
7. A. Mina (2020) Comparison of several immunoassays used in drugs of abuse screening: Assessment against gold standard methods and calculation of measurement uncertainty. *J Pharmacol Toxicol Methods* 101: 106649.
8. R. Wijngaard, M. Parra-Robert, L. Marés, A. Escalante, E. Salgado et al. (2021) Tetracaine from urethral ointment causes false positive amphetamine results by immunoassay. *Clin Toxicol (Phila)* 59(6): 500-505.
9. M. Leclercq, M. Soichot, B. Delhotal-Landes, E. Bourgogne, H. Gourlain, et al. (2020) False positive amphetamines and 3,4-methylenedioxymethamphetamine immunoassays in the presence of metoprolol-two cases reported in clinical toxicology. *J Anal Toxicol* 44(2): 200-205.
10. A. Begeman, E.J.F. Franssen (2018) Lack of Detection of New Amphetamine-Like Drugs Using Conventional Urinary Immunoassays. *Ther Drug Monit* 40(1): 135-139.
11. S. Bugier, C. Garcia-Hejl, P. Vest, J. Plantamura, D. Chianea, et al., (2016) A Cross-Reactivity of Fenofibric Acid with MDMA DRI Assay. *Mil Med* 181(9): 1013-1015.
12. L. Quesada, I. Gomila, A. Fe, M.A. Servera, C. Yates, D. Morell-Garcia, et al., (2015) Fenofibric Acid Can Cause False-Positive Urine Methylenedioxymethamphetamine Immunoassay Results. *J Anal Toxicol* 39(9): 734-740.
13. Y.C. Kaplan, A. Erol, B. Karadaş (2012) False-positive amphetamine/ecstasy (MDMA/3,4-methylenedioxymethamphetamine) (CEDIA) and ecstasy (MDMA/3,4-methylenedioxymethamphetamine) (DRI) test results with fenofibrate. *Ther Drug Monit* 34(5): 493-495.
14. B.K. Logan, A.G. Costantino, E.F. Rieders, D. Sanders (2010) Trazodone meta-chlorophenylpiperazine (an hallucinogenic drug and trazodone metabolite), and the hallucinogen trifluoromethylphenylpiperazine cross-react with the EMIT®II ecstasy immunoassay in urine. *J Anal Toxicol* 34(9): 587-589.