Legal Medicine 17 (2015) 499–502

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Short Communication

Evaluation of the on-site immunoassay drug-screening device Triage-TOX in routine forensic autopsy

Mariko Tominaga, Tomomi Michiue *, Hitoshi Maeda

Department of Legal Medicine, Osaka City University Medical School, Asahi-machi 1-4-3, Abeno, Osaka 545-8585, Japan
Forensic Autopsy Section, Medico-legal Consultation and Postmortem Investigation Support Center, c/o Osaka City University Medical School, Asahi-machi 1-4-3, Abeno, Osaka 545-8585, Japan

ARTICLE INFO

Article history:
Received 4 March 2015
Received in revised form 8 October 2015
Accepted 21 October 2015
Available online 23 October 2015

Keywords:
Forensic toxicology
On-site immunoassay drug screening
Gas chromatography/mass spectrometry
Liquid chromatography/tandem mass spectrometry
Urine
Pericardial fluid

ABSTRACT

Instrumental identification of drugs with quantification is essential in forensic toxicology, while on-site immunoassay urinalysis drug-screening devices conveniently provide preliminary information when adequately used. However, suitable or sufficient urine specimens are not always available. The present study evaluated the efficacy of a new on-site immunoassay drug-screening device Triage-TOX (Alere Inc., San Diego, CA, USA), which has recently been developed to provide objective data on the one-step automated processor, using 51 urine and 19 pericardial fluid samples from 66 forensic autopsy cases, compared with Triage-Drug of Abuse (DOA) and Monitect-9. For benzodiazepines, the positive predictive value and specificity of Triage-TOX were higher than those of Triage-DOA; however, sensitivity was higher with Monitect-9, despite frequent false-positives. The results for the other drugs with the three devices also included a few false-negatives and false-positives. These observations indicate the applicability of Triage-TOX in preliminary drug screening using urine or alternative materials in routine forensic autopsy, when a possible false-negative is considered, especially for benzodiazepines, providing objective information; however, the combined use of another device such as Monitect-9 can help minimize misinterpretation prior to instrumental analysis.

1. Introduction

Systematic instrumental identification of drugs with quantification is essential in forensic and clinical toxicological analysis, while on-site immunoassay urinalysis drug-screening devices have advantages regarding their economic and technical convenience as well as short turn-around-time performance. In forensic toxicology, on-site urinalysis drug screening is helpful in providing preliminary information on several drugs of abuse when adequately used in consideration of possible false-negatives or -positives [1–8]; however, suitable or sufficient urine specimens are not always available. Thus, a device that can be used with a minimum amount of specimen, including other body fluids, is preferable. Another issue in previous on-site immunoassay screening is poor objectivity in cases of obscure positivity, owing to the observer's visual reading. Meanwhile, the on-site drug-screening device Triage-TOX (Alere Inc., San Diego, CA, USA), using competitive fluorescence immunoassay, was recently developed to provide preliminary qualitative results through one-step processing after sample application to the automatic analyzer, ensuring objectivity by instrumental colorimetric calibration followed by the printing of positive/negative results, independent of the operator [9,10]; however, there have been limited published data establishing the efficacy of this device in forensic autopsy cases.

Against the aforementioned background, the present study evaluated the efficacy of the on-site immunoassay drug-screening device Triage-TOX, which can present objective data on the one-step automated processor, using urine and pericardial fluid specimens from forensic autopsy cases without evident decomposition, compared with Triage-Drug of Abuse (DOA) and Monitect-9.

2. Materials and methods

2.1. Materials

Urine (n = 51) and pericardial fluid (PCF, n = 19) samples, stored at −20 °C until use, were collected from 66 forensic autopsy cases without evident putrefactive changes due to decomposition (January 2011–August 2014) to include cases where a spectrum of the following common drugs were detected by instrumental

* Corresponding author at: Department of Legal Medicine, Osaka City University Medical School, Asahi-machi 1-4-3, Abeno, Osaka 545-8585, Japan.
E-mail address: michi.leg@med.osaka-cu.ac.jp (T. Michiue).

http://dx.doi.org/10.1016/j.legalmed.2015.10.007
1344-6223/

analysis as described below [11,12]: Urine samples contained benzodiazepines (BZO, $n = 24$), including alprazolam ($n = 3$), diazepam ($n = 2$), estazolam ($n = 2$), etizolam ($n = 6$), flunitrazepam ($n = 2$), nitrazepam ($n = 6$), triazolam ($n = 3$) and their metabolites ($n = 8$), barbiturates (BAR, $n = 14$; phenobarbital), amphetamine (AMP, $n = 9$), methamphetamine (MET, $n = 8$), tricyclic antidepressants (TCA, $n = 6$); clomipramine ($n = 3$) and imipramine ($n = 3$), and acetaminophen (APAP: $n = 3$), and PCF contained BZO ($n = 13$), including bromazepam ($n = 1$), clotiazepam ($n = 3$), diazepam ($n = 3$), estazolam ($n = 1$), nitrazepam ($n = 4$), triazolam ($n = 3$) and their metabolites ($n = 11$), BAR ($n = 2$; phenobarbital), AMP ($n = 2$), MET ($n = 1$) and TCA ($n = 1$; imipramine). All specimens were negative for phencyclidine (PCP), cocaine (COC), tetrahydrocannabinol (THC), opiates (OPI) and methadone (MTD).

2.2. On-site immunoassay drug screening

Triage-TOX (Alere Inc., San Diego, CA, USA) and two reference devices, Triage-DOA (Sysmex Inc., Kobe, Japan) and Monitect-9 (VERITAS, Tokyo, Japan), were used [13,14]. Triage-TOX devices and the automated analyzer Alere Triage Meter Pro (San Diego CA, USA) were provided by Sysmex Corporation (Kobe, Japan) for the present study [15]. Minimum required amounts of specimens were 240 μg/mL for Triage-TOX, 140 μg/mL for Triage-DOA and 750 μg/mL for Monitect-9. The abbreviations and threshold urine concentrations are as listed in the manufacturers' instructions (Supplementary Table 1) [10,13,14]. These devices were used following the manufacturers' instructions, which were also applied to PCF analysis. This study was approved by the institutional conflict-of-interest and ethics committees.

2.3. Instrumental analysis

Gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/tandem mass spectrometry (LC/MS/MS) were used for instrumental identification and quantification of drugs, as previously reported [11,12]. Detection limits (cutoff values) were 0.01–0.05 μg/mL for BZO and its metabolites, 0.01 μg/mL for BAR, 0.001 μg/mL for AMP and MET, 0.01 μg/mL for TCA, 0.001 μg/mL for OPI, and 0.001 μg/mL for APAP with GC/MS; and 0.001–0.005 μg/mL for BZO and metabolites, 0.001 μg/mL for BAR, 0.001 μg/mL for MET, 0.001–0.005 μg/mL for TCA, and 0.001 μg/mL for OPI with LC/MS/MS. After drug screening using both methods, quantitative analysis was performed primarily using GC/MS, followed by LC/MS/MS for BZO, diazepam metabolites (nordiazepam, oxazepam, and temazepam), and TCA (nortriptiline), which were not detected by GC/MS.

2.4. Definitions

A 'false-positive' was recorded when the on-site screening device indicated a positive result but no target drug or metabolite was detected by instrumental analysis, while a 'false-negative' was defined as a negative result with the device using the sample containing the target drug or metabolite over the threshold concentration [8].

3. Results and discussion

Substantial numbers of urine and PCF samples were available for BZO to evaluate positive and negative findings on Triage-TOX and the two reference devices in the present study (Tables 1 and 2, as well as Supplementary Tables 2 and 3); sensitivity and specificity were substantially different among these devices despite similar threshold levels of BZO mentioned in the manufacturers' instructions [10,13,14]. Among BZO-positive cases with Triage-TOX and Triage-DOA, three urine and two PCF samples contained diazepam (0.006–0.334 μg/mL) with its metabolites: nordiazepam (0.025–1.414 μg/mL) in three urine and two PCF sample, oxazepam (0.003–2.344 μg/mL) in three urine and one PCF sample, and temazepam (0.005–4.013 μg/mL) in three urine samples. Metabolite concentrations were higher, except for in a PCF sample (diazepam, 0.334 μg/mL), suggesting the major contribution of metabolites to positivity. Meanwhile, a few urine and PCF samples containing BZO or its derivatives above the reference minimum-detection-limit concentration (0.334–9.079 μg/mL) resulted in false-negatives [10,13,14,16] (Supplementary Table 4). These false-negative cases included four urine samples (temazepam, 0.687 μg/mL; flunitrazepam, 0.738 μg/mL; lorazepam, 1.07 μg/mL; and nordiazepam, 9.079 μg/mL) with Triage-TOX, and three urine samples

Table 1
Positive predictive value (PPV) and negative predictive value (NPV) in drug screening using postmortem urine with Triage-TOX, Triage-DOA and Monitect-9 as estimated based on the threshold concentrations listed in the manufacturer's instructions [13–15].

Urine		Triage-TOX (%)	Triage-DOA (%)	Monitect-9 (%)
Benzodiazepines	PPV	55.6	66.7	100
	NPV	73.8	100	45.2
Barbiturates	PPV	92.3	100	76.9
	NPV	94.7	94.7	92.1
Amphetamine	PPV	71.4	85.7	57.1
	NPV	95.5	95.5	97.7
Methamphetamine	PPV	100	–	100
	NPV	93.2	–	95.5
Tricyclic antidepressants	PPV	50.0	75.0	100
	NPV	95.8	95.7	80.9
Tetrahydrocannabinoid	PPV	–	–	–
	NPV	100	100	92.2
Opioids	PPV	–	–	–
	NPV	94.1	94.1	100
Acetaminophen	PPV	100	–	–
	NPV	93.9	–	–

Table 2
Positive predictive value (PPV) and negative predictive value (NPV) in drug screening using postmortem pericardial fluid with Triage-TOX, Triage-DOA and Monitect-9 as estimated based on the threshold concentrations listed in the manufacturer's instructions [13–15].

Pericardial fluid		Triage-TOX (%)	Triage-DOA (%)	Monitect-9 (%)
Benzodiazepines	PPV	60	40	100
	NPV	100	100	35.7
Barbiturates	PPV	100	50	100
	NPV	100	100	100
Amphetamine	PPV	–	–	–
	NPV	100	100	100
Methamphetamine	PPV	–	–	–
	NPV	100	–	100
Tricyclic antidepressants	PPV	–	–	–
	NPV	100	100	100
Tetrahydrocannabinoids	PPV	–	–	–
	NPV	100	100	100
Opioids	PPV	–	–	–
	NPV	100	100	100
Acetaminophen	PPV	–	–	–
	NPV	100	–	–