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Significantly increased detection rate of drugs of abuse in urine following the introduction of new German driving licence re-granting guidelines☆

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ABSTRACT

In this paper we present the first assessment of the new German driving licence re-granting medical and psychological assessment (MPA) guidelines by comparing over 3500 urine samples tested under the old MPA cut-offs to over 5000 samples tested under the new MPA cut-offs. Since the enzyme multiplied immunoassay technique (EMIT) technology used previously was not sensitive enough to screen for drugs at such low concentrations, as suggested by the new MPA guidelines, enzyme-linked immunosorbent assay (ELISA) screening kits were used to screen for the drugs of abuse at the new MPA cut-offs. The above comparison revealed significantly increased detection rates of drug use or exposure during the rehabilitation period as follows: 1.61, 2.33, 3.33, and 7 times higher for 11-nor-delta-9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH), morphine, benzoylecgonine and amphetamine respectively. The present MPA guidelines seem to be more effective to detect non-abstinence from drugs of abuse and hence to detecting drivers who do not yet fulfil the MPA requirements to regain their revoked driving licence.

1. Introduction

The medical and psychological assessment (MPA) was established in 1955 and since then is claimed to be a successful tool for the diagnosis and advancement of the fitness to drive [1,2]. "Those persons considered fit for operating a motor vehicle must meet the necessary physical and mental requirements and may not have severely or repeatedly transgressed against traffic regulations or penal law" [1]. Hence, MPA has an interdisciplinary approach, integrating medical, psychological and toxicological criteria. In Germany the most common reasons for revoking the driving licence and requesting an MPA are traffic violations under the influence alcohol, either after the first incident involving alcohol at 1.6 per mille or higher, or after repeated incidents of driving while intoxicated with alcohol or drugs of abuse. The latter is defined in § 24a StVG ("Straßenverkehrsgesetz", German Road Traffic Act) as driver's drug concentration in blood higher than 1 ng/mL for THC and/or 10 ng/mL for morphine and/or 25 ng/mL for amphetamines or designer amphetamines and/or 10 ng/mL for cocaine and/or 75 ng/mL for benzoylecgonine. As part of the driving licence re-granting process, such ex-drivers have to prove complete abstinence for twelve months [1]. In the case of drugs of abuse this is done generally by six "random" (24 h notice) urine tests in twelve months and recently also by hair analysis covering a calendar year. In this way, the MPA offers increased traffic safety on the one hand by identifying unfit dangerous drivers and removing them from traffic until they restore their fitness to drive. On the other hand the MPA helps the unfit drivers to rehabilitate themselves. Already in 1999, the recidivism rate was stated to have dropped by more than 50% [3].

In 2009 the criteria used for the MPA to judge if a person is fit to drive in order to regain a revoked driving licence were revised by the German Society of Traffic Psychology and German Society of Traffic Medicine [4]. The revision entered into force on the 1st July 2009 and includes a polytoxicological drug screening in urine and/or hair using a zero-limit approach to assess abstinence including benzodiazepines and methadone; ex-heroin abusers are also tested for opioids buprenorphine, tilidine, tramadol and their metabolites. The aim of this paper is to compare the rate of confirmed positive samples for the drugs of abuse – opiates, cocaine, cannabinoids and amphetamines, using a statistically significant population before and after the introduction of new guidelines and hence to assess their efficacy in detecting non-abstinence.

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2. Methods

2.1. Analytical procedures

The drug screenings tested according to the old MPA guidelines were performed using CE labelled enzyme multiplied immunoassay technique (EMIT) by Siemens Healthcare Diagnostics run on a Dade Dimension Xpand Autoanalyser System. The EMIT methods were validated by the supplier as follows: 115, 125, 203 and 129 urine specimens were tested with the OPI, COC, THC and AMPH flex® cartridge respectively on the Dimension® system at 300 ng/mL for opiates, 300 ng/mL for benzoylecgonine, 50 ng/mL for cannabinoids and 500 ng/mL for amphetamines and with the respective method on the Syva®-30R Biochemical System (comparative method) using the Syva® Emit® II Plus Opiates, Cocaine, Cannabinoids and Amphetamines assay respectively; subsequently the samples were tested by gas chromatography–mass spectrometry (GC–MS) as a reference method. At our laboratory, the EMIT screening tests have been accredited to DIN EN ISO/IEC 17025 since 2001. Furthermore, we partially validated the EMIT tests for all substance classes by determining the intraassay and interassay imprecision, bias and correlation coefficient with the confirmation method. For all the above mentioned four drug classes of abuse, the imprecision and bias were less than 15% and the correlation coefficient with GC–MS measurements greater than 0.96 as required by our quality management system.

The screenings for drugs of abuse in urine tested according to the new MPA guidelines, were performed using CE labelled enzyme-linked immunosorbent assay (ELISA) screening kits from nal von Minden GmbH (Regensburg, Germany) run on a BEP 2000 Advance® System from Siemens Healthcare Diagnostics. Essentially, the LUCIO®-Direct ELISA kits were validated for the urine matrix at the new MPA guidelines' cut-offs by confirming at least 100 authentic samples including both positive and negative samples by GC–MS, following ELISA screening. For drugs for which at least 5% positive samples were present, namely THC-COOH, benzoylecgonine, amphetamine, morphine and codeine, Response Operating Curve (ROC) analysis was performed. ELISA cut-offs were chosen at the point on the ROC curve corresponding to less than 1% false negatives. For each of the other drugs for which no authentic positive urine samples were available, namely methamphetamine, 3,4-methylenedioxyamphetamine, (MDA), 3,4-methylenedioxy-N-methamphetamine, (MDMA) and methylendioxy-N-ethylamphetamine, (MDE), dihydrocodeine and 6-monoacetylmorphine, (6-MAM), five blank urine samples were spiked at the required concentration as stated by the new MPA guidelines were measured. The lower ELISA value was chosen as the drug class cut-off, thus ensuring the effective detection of all the 12 drug substances shown in Table 1 at the required new MPA concentrations. The sensitivities and specificities for the chosen ELISA cut-off for amphetamines, cannabinoids, cocaine and opiates are shown in Table 1 at the chosen screening cut-off. Prior to the screening and confirmation of THC-COOH, the sample was hydrolyzed by addition of 20 μL 10 M NaOH to 0.5 mL urine sample mixed and heated for 30 min at 80 °C. Similarly, opiates were hydrolyzed with the addition of 30 μL β-glucuronidase/arylsulfatase mix and incubated for 2 h at 55 °C. A separate publication [5] describes in detail the validation of the LUCIO®-Direct ELISA kits for the urine matrix at the new MPA guidelines' cut-offs. The confirmation of positive screening tests were done using GC–MS after sample preparation using solid phase extraction (SPE) cartridges and appropriate derivatisations [6,7]. The ELISA screening tests and the GC–MS confirmation methods were re-accredited to DIN EN ISO/IEC 17025 *for forensic purposes* as required by the new MPA guidelines [4].

3. Results and discussion

Before the introduction of the new guidelines, in the period from January to June 2009, 3536 urine samples were screened for drugs of abuse using EMIT at the suppliers' cut-offs used worldwide for clinical and forensic cases [8–11]. These cut-offs will be referred to as "conventional cut-offs" or "old MPA cut-offs" throughout this paper and are shown in Table 2.

In the period from July to December 2009, 5058 urine samples were screened for drugs of abuse as defined in the new guidelines shown in Table 2 using ELISA technique. In both cases, positively screened samples were confirmed by GC–MS.

3.1. Comparing positive samples using old and new MPA cut-offs

374 out of 5058 urine samples (7.4%) were confirmed positive for amphetamines, cannabinoids, cocaine or opiates following ELISA screening at the new MPA cut-offs as opposed to 124 out of 3536 urine samples (3.5%) using EMIT screening at the conventional cut-offs. Considering each drug class separately, the number of confirmed positive samples increased from 95 (2.6%) to 214 (4.2%) for THC-COOH, from 12 (0.3%) to 36 (0.7%) for morphine

Table 1
Summary of the screening validation results for target drug analytes; the underlined values in bold show the chosen screening cut-offs for the respective drug class.

Substance	ROC analysis data					Confirmation data			Spiked substances' data			
	Chosen screening cut-off in A.U.	Sensitivity	Specificity	Youden Index	No. of samples	Confirmation cut-off in ng/mL	LOD in ng/mL	LOQ in ng/mL	No. of true positives	Average ELISA value of spiked substance at confirmation cut-off	Variation coefficient of spiked substance at confirmation cut-off	% Cross-reactivities
THC-COOH	**<u>20</u>**	97%	69%	0.66	108	10.0	2.5	8.8	22			100%
Cocaine							0.3	2.5				70%
Benzoylecgonine	**<u>73</u>**	100%	93%	0.83	102	30.0	1.2	17.1	9			100%
Amphetamine	**<u>80</u>**	94%	51%	0.45	105	50.0	9.3	25.5	18			100%
MDA						50.0	8.2	44.2	0	281	1%	250%
Metamphetamine						50.0	7.6	14.0	1	86	10%	100%
MDMA						50.0	9.4	27.9	0	**<u>79</u>**	5%	135%
MDE						50.0	3.5	27.1	0	<u>17</u>	4%	10%
Dihydrocodeine						25.0	3.6	7.1	0	31	15%	85%
Codeine	40	100%	81%	0.76	100	25.0	2.0	9.5	5			200%
Morphine	**<u>31</u>**	100%	83%	0.83	100	25.0	2.1	3.2	10			100%
6-MAM						25.0	2.1	4.6	0	13	11%	83%