Forensic abstinence control with KIMS immunoassays for drugs of abuse and ethyl glucuronide in urine on a cobas c 501 analyzer

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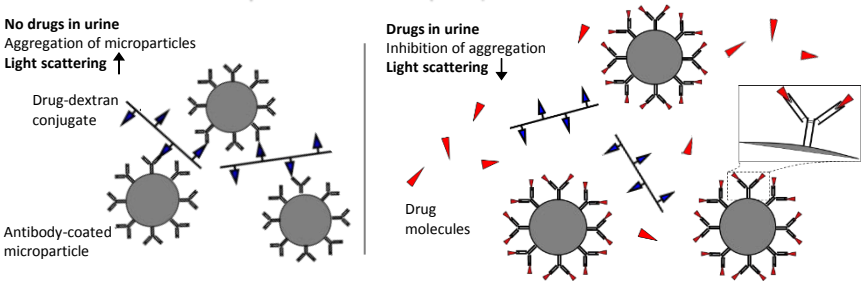
Introduction

Roche cobas c501

For the **medico-psychological assessment (MPA)** during driving license re-granting in Germany, abstinence control including urine samples is required. In these programs, even small amounts of markers for drug or alcohol abuse have to be detected. Thus, **the concentrations of the target compounds are very low**, and, in consequence, the sensitivity of the applied screening method has to be much higher than for clinical use. Modified drugs of abuse and ethyl glucuronide immunoassays were evaluated for **precision, accuracy, onboard calibration stability, cross reactivity, sensitivity and specificity**.

Experimental

Kinetic interaction of microparticles in solution (KIMS)



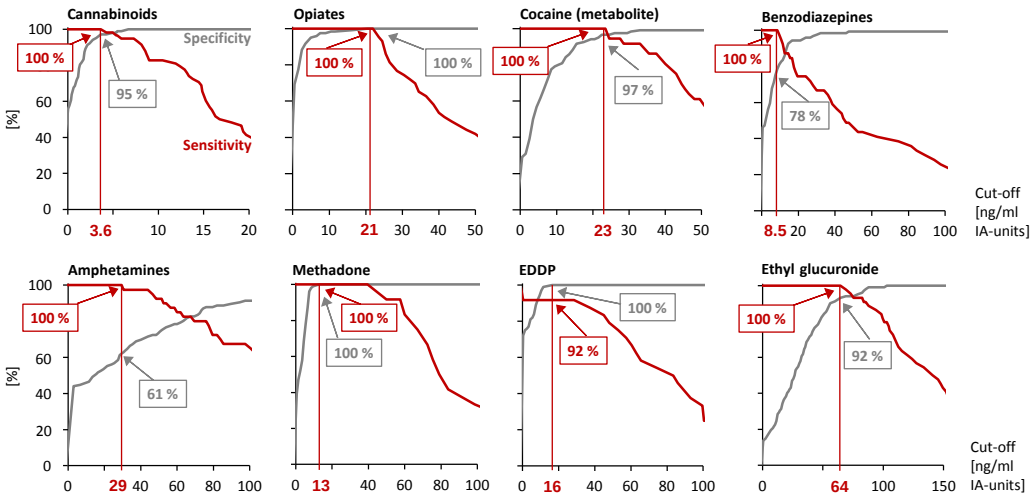
Parameters

IA reagents, target analytes and MPA limits (ng/ml urine)

Cannabinoids (THC2) THC-COOH **10** (after hydrolysis)
Opiates (OPI2) morphine (codeine, dihydrocodeine) **25** (after hydrolysis)
Cocaine (COC2) benzoylecgonine **30**
Amphetamines (AMPS2) amphetamine, methamphetamine, MDMA, MDEA, MDA **50**
Methadone (MDN2, MM (EMIT-Assay)) EDDP **50**
Benzodiazepines (BNZ2) diazepam, nordazepam, oxazepam, hydroxy-alprazolam, hydroxybromazepam, 7-aminoflunitrazepam, lorazepam **50**
Ethyl glucuronide (ETG (DRI-Assay)) **100**

Results

Sensitivity, Specificity and Cut-offs



For **sensitivity and specificity**, the immunoassay values of at least 100 negative and 50 positive samples per substance class* were compared with the quantitative LC–MS/MS or GC–MS values using ROC (receiver operating characteristic) analysis. At least 10 positive samples for each of the MPA-target analytes were included. *methadone: 22, EDDP: 28 positive samples. For the sensitivity the result of the confirmatory analysis was regarded as positive if the measured concentration reached or exceeded a concentration of 60% of the respective MPA limit (supposed measurement uncertainty 40%).

Quality controls, Precision, Accuracy

	Target Concentration [ng/ml]			Inter-day (RSD, %)			Inter-day (bias, %)		
	Low	Middle	High	Low	Middle	High	Low	Middle	High
Cannabinoids	8	12.5	30	8.5	6.2	5.6	-4.4	4.3	4.7
Opiates	22	30	225	4	2.8	2.3	1	12	0.5
Cocaine (metabolite)	22.5	37.5	225	5.9	3.5	1.9	2	-0.8	9.7
Amphetamines	37.5	62.5	375	5.6	4.4	3.4	5.9	5.6	-6
EDDP	37.5	62.5	250	5.7	3.8	n.d.	1.5	1.4	n.d.
Methadone	37.5	62.5	375	3.2	2.8	3.7	9.2	11	4.1
Benzodiazepines	37.5	62.5	250	4.6	2.7	1.2	-4.6	-2.3	1.5
Ethyl glucuronide	75	125	1250	6	3.9	1.4	1	1.3	-3.8

Calibration stability: 28 days for all analytes except amphetamines (21 days)

For onboard **calibration stability**, the low and middle quality control samples were analyzed with n = 5 repetitions at day 0 and with n = 3 repetitions at day 7, 14, 21, and 28.

Inter-day **precision and accuracy** were determined with n = 20 repetitions each at five consecutive days.

For **cross reactivity**, a Roche negative calibrator was spiked with one of the analytes at concentrations yielding immunoassay values within the calibration range of the assay.

Cross reactivity

Cannabinoids	%
9-Carboxy-11-nor-Δ9 THC-glucuronide	34
11-Hydroxy-Δ9 THC	14
Opiates	
Morphine-3-glucuronide	38
Codeine	99
6-Acetylmorphine	71
Diacetylmorphine	68
Codeine-6-glucuronide	61
Dihydrocodeine	55
Morphine-6-glucuronide	47
Dihydromorphine	38
Thebaine	34
Hydrocodone	22
Hydromorphone	19
Cocaine (metabolite)	
Cocaine	0.94
Ecgoninmethylester	0.003
Amphetamines (and NPS)	
6-(2-Aminopropyl)-benzofurane	150
± MDMA	130
± MDA	110
4-Fluoromethamphetamin	92
5-(2-Aminopropyl)-benzofurane	87
± MBDB-HCl	68
4-Fluoroamphetamin	54
Amphetamine	46
± MDEA	43
Methylphenidate	0.31
Methadone	
Levomepromazine	4.3
EDDP	1.8
EDDP	
Methadone	0.033
Benzodiazepines	
Bromazepam	110
α-Hydroxyalprazolam	92
Flubromazolam	91
Oxazepam	76
α-Hydroxybromazepam	75
Lorazepam	70
7-Aminoflunitrazepam	66
Ethyl glucuronide	
Ethyl sulfate	0.011

Conclusion

The presented kinetic interaction of microparticles in a solution (KIMS) immunoassays on a **cobas c** 501 provide a new method to reliably detect drug or alcohol consumption in abstinence control programs requiring high sensitivity.

Literature

M.A. Neukamm, A. Bahrami, V. Auwärter, F.M.P. Mehne, E. Höss: Evaluation of KIMS immunoassays on a **cobas c** 501 analyzer for drugs of abuse and ethyl glucuronide testing in urine for forensic abstinence control, *Drug Testing and Analysis* 2017, DOI 10.1002/dta.2154

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