"United Forces – Drugs of Abuse"
Immunoassay based screening (LUCIO® Drug ELISA) for drugs of abuse using the Greiner Bio-One Saliva Collection System (SCS)

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Introduction

Oral fluid (OF) is a matrix for drugs of abuse testing which can be used to elucidate whether someone is under the influence, and if the cut-off is chosen wisely, could replace urine screenings in the future. Here we present an aligned solution for immunological OF screening. "United Forces" combines the liquid based Saliva Collection System (SCS), which allows easy and fast oral fluid collection, with ELISA (_enzyme-linked immunosorbent assay_) screening of seven different drugs of abuse (amphetamine, methamphetamine, cocaine, benzodiazepines, opiates, methadone, THC). In the focus of this work were two different settings with different requirements each: in the clinical setting like rehabilitation centers, the requirements for saliva aim towards the replacement of urine and thus needs very sensitive measurements. On the other hand in the workplace drug testing environment (EWDTS guidelines), saliva has to answer the question whether a proband is currently under the influence of any drug.

Methods

Patient samples (kindly provided by Dr. Michael Böttcher, MVZ Labor Dessau GmbH): All OF samples (n=420) were randomly taken during a routine period of two weeks. All samples came from patients in heroin substitution therapy. OF samples were collected with SCS, the OF concentration was photometrically determined and the drug screening was initially performed with LC/MS-MS (cut off (CO): 1 ng/ml). LUCIO® Drug ELISA: The screening cut offs (CO) were optimized via Receiver Operating Characteristic (ROC) analysis to reach highest sensitivity and specificity for all tests depending on the required/recommended confirmation CO. Samples were then measured with four level multi calibration standards. The levels were adapted to the respective CO and analyzed in duplicates. Two different settings were analyzed: in the clinical setting a LC/MS CO of 5 ng/ml was exemplarily selected. In the setting "workplace drug testing" COs followed EWDTS guidelines.

Conclusion

1. LUCIO® Drug ELISAs are a suitable tool to screen OF samples collected with the Greiner Bio-One SCS for drugs of abuse
2. The United Forces testing workflow can be used for both examples: clinical screenings and workplace drug testing. Higher false negative rates within the clinical setting are usually compensated by more frequent testing.
3. Different screening COs are needed to meet the requirements of different settings.

Results:

Clinical setting

Table 1: example (Opiates) of cross table for the evaluation of LUCIO® Drug ELISAs in a clinical setting

			LC/MS - Cut-Off: 5 ng/ml	
			Pos	Neg
			38	382
LUCIO® Drug ELISA Cut-Off: 6 ng/ml	Pos	40	**True Positive** 37	**False Positive** 3
	Neg	380	**False Negative** 1	**True Negative** 379
n=420			**Sensitivity** 97,4 %	**Specificity** 99,2%
Positive rate LC/MS:	9,0		**PPV*** 92,5%	**NPV**** 99,7%
Positive rate ELISA:	9,5			

Workplace drug testing

Table 2: example (Opiates) of cross table for the evaluation of LUCIO® Drug ELISAs in a setting for worplace drug testing

			LC/MS - Cut-Off: 20 ng/ml	
			Pos	Neg
			26	394
LUCIO® Drug ELISA Cut-Off: 20 ng/ml	Pos	28	**True Positive** 26	**False Positive** 2
	Neg	392	**False Negative** 0	**True Negative** 392
n=420			**Sensitivity** 100%	**Specificity** 99,5%
Positive rate LC/MS:	6,2		**PPV*** 92,9%	**NPV**** 100%
Positive rate ELISA:	6,7			

*PPV: positive predictive value
**NPV: negative predictive value

Table 3: Summary of all evaluation data of LUCIO® Drug ELISAs in a clinical setting

Parameter	ELISA CO[#] [ng/ml]	LC/MS CO[#] [ng/ml]	True Pos	True Neg	False Pos	False Neg	Pos rate LC/MS [%]	Pos rate ELISA [%]	PPV* [%]	NPV** [%]	Sensitivity [%]	Specificity [%]
Amphetamine	8	5	28	383	2	7	8,3	7,1	93,3	98,2	80,0	99,5
Methamphetamine	20	5	27	381	3	9	8,6	7,1	90,0	97,7	75,0	99,2
THC	2,5	5	73	317	14	7	19,0	20,7	83,9	97,8	91,3	95,8
Opiates	6	5	37	379	3	1	9,0	9,5	92,5	99,7	97,4	99,2
Cocaine	15	5	18	394	3	5	5,5	5,0	85,7	98,7	78,3	99,2
Benzodiazepines	10	5	36	370	12	1	9,0	11,4	75,0	99,5	94,7	96,9
Methadone	10	5	256	145	8	11	63,6	62,9	97,0	92,9	95,9	94,8

Table 4: Summary of all evaluation data of LUCIO® Drug ELISAs in a setting for workplace drug testing

Parameter	ELISA CO[#] [ng/ml]	LC/MS CO[#] [ng/ml]	True Pos	True Neg	False Pos	False Neg	Pos rate LC/MS [%]	Pos rate ELISA [%]	PPV* [%]	NPV** [%]	Sensitivity [%]	Specificity [%]
Amphetamine	10	20[##]	17	395	6	2	4,5	5,5	73,9	99,5	89,5	98,5
Methamphetamine	20	20[##]	22	390	8	0	5,2	7,1	73,3	100	100	98
THC	1,5	2	88	309	15	8	23,3	24	85,4	97,5	91,7	95,4
Opiates	20	20	26	392	2	0	6,2	6,7	92,9	100	100	99,5
Cocaine	15	8	18	397	3	2	4,8	5	85,7	99,5	90	99,3
Benzodiazepines	10	5	36	370	12	1	9,0	11,4	75,0	99,5	94,7	96,9
Methadone	10	20	252	145	12	11	62,6	62,9	95,5	92,9	95,8	92,4

[#]: CO values refer to neat saliva
[##] CO differs from EWDTS recommendations due to different LC/MS measurement frame