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From: Alain Verstraete <alain.verstraete@ugent.be>
To: <wvogl@samhsa.gov>
Date: 7/12/04 1:41PM
Subject: Comment on SAMHSA, HHS docket number 04-7984

Dear Dr. Vogl,

Here are a few comments on the proposed revisions to mandatory guidelines for federal workplace drug testing programs.
- one issue is the necessity to collect oral fluid be spitting, which is not very hygienic. Samyn et al. have shown (JFS 2002; 47: 1380, = reference 53, on page 19689 of the Federal Register) that for THC, the concentrations measured after sampling with a Salivette are much higher than with spitting. Our hypothesis is that THC sticks to the gingival mucosa and gets adsorbed to the cotton roll rather than be dissolved in the aqueous fluid of the oral cavity. This is probably also the case with other devices where the collection process involves wiping the tongue or the mouth mucosa. For POCT and ELISA tests, manufacturers have developed specific collection devices that are optimized for use with their detection system. If these systems have to be used with oral fluid collected by spitting, for which they were not optimized, their performance might be worse. In addition to spitting, an FDA approved collection device should also be allowed.
- another major issue is the requirement that a urine sample is collected every time an oral fluid sample is collected. This requirement negates the advantages of oral fluid drug testing (less invasive sample collection and expected correlation with impairment because of the shorter detection window). Under these circumstances, it is doubtful that many agencies will be inclined to use oral fluid testing. I do understand the concerns about environmental contamination, and I do hope that enough data will become available soon, so the requirement for the extra urine sample can be dropped.
- in our experience, immunoassays for methamphetamine work very well for the detection of MDMA, and only one immunoassay is sufficient. I attach the text of our abstract, to be presented at the TIAFT-SOFT meeting in Washington early September (for the comparison the EU recommended confirmation cut-off of 200 ng/mL was used, but there will be few changes if it is increased to 250 ng/mL).See e.g. the results from the first Rosita study with POCT tests, where a combination of an amphetamine and methamphetamine test (no MDMA tests were available at the time) to detect amphetamine and/or MDMA in urine resulted in an accuracy of >= 98% for 3 out of 6 manufacturers.
- on page 19697, in the second table under section 3.5, phencyclidine is mentioned under the opiates, which is not correct; it should be aligned more to the left. Idem for the second table under section 3.7
- on page 19719, under section 12.9 (i), the units for IgG in oral fluid are missing

With best regards,

Prof. Dr. Alain Verstraete
Laboratory of Clinical Biology - Toxicology
Ghent University Hospital
De Pintelaan 185
B-9000 Gent Belgium
Phone + 32 9 240 34 07
Fax + 32 9 240 49 85

Comparison of the sensitivity and specificity of six immunoassays for the detection of amphetamines.

Alain Verstraete, Fien Vander Heyden
Laboratory of clinical chemistry, section toxicology, Ghent University Hospital, De Pintelaan 185, B-9000 Gent

Introduction

In drug of abuse screening, the ideal amphetamine immunoassay should detect amphetamine and the different illicit amphetamine analogues (e.g. MDMA, MDEA, MDA) without false positive results from anorectics, other stimulants or other drugs like ranitidine. We compared the sensitivity and specificity of 6 commercial urine amphetamine immunoassays for the analysis of the urine samples that were sent to our laboratory during a 2.5-month period.

Methods

Two hundred twenty five urine samples that had been sent to our laboratory for screening or confirmation of amphetamines were analyzed for amphetamines with the FPIA amphetamine/methamphetamine II assay (on Abbott ADx and AxSYM instruments), EMIT (Emit II Plus Monoclonal Amphetamine/Metamphetamine Assay and the new EMIT II Plus Amphetamines Assay) and KIMS (standard protocol and MDMA protocol, KIMS and KIMS X respectively). All assays were calibrated and used semi-quantitatively.
All samples that screened positive by any amphetamine screening method and 15% of the negative samples were confirmed by LC-MS/MS. Briefly, 10 µL of urine was mixed with 90 µL of a mixture of deuterated internal standards and 20 µL was injected in the LC-MS/MS. The assay LOQ is less than 15 ng/mL of amphetamine, methamphetamine, MDMA, MDEA, MDA, 4-MTA and PMA. A sample was considered positive for amphetamines if any of these substances was present at > 200 ng/mL.

Results and discussion

Ninety-one (40%) of the samples were positive by LC-MS/MS. The number of positive samples, lowest, median and highest concentration (in ng/mL) are 74, 71, 2560 and 155000 for amphetamine, 1, 33, 33 and 33 for methamphetamine, 27, 46, 5975 and 108000 for MDMA, 23, 15, 516, 12400 for MDA and 4, 27, 1530 and 24800 for MDMA. MBDB, 4-MTA and PMA were not found.

Some characteristics of the different assays are given in the table:

	ADx	AxSYM	EMIT	EMIT N	KIMS	KIMS X
Area under the ROC curve	0.999	0.988	0.977	0.984	0.975	0.972
95% confidence interval	0.982-1.000	0.963-0.998	0.948-0.993	0.958-0.996	0.944-0.991	0.941-0.990
Optimal cut-off (ng/mL)	350	677	565	271	404	493
Sensitivity at the cut-off (%)	98.9%	95.6%	96.6%	90.9%	94.4%	93.3%
Specificity at that cut-off (%)	98.5%	97.8%	90.2%	100%	88.5%	89.3%
# false negatives at that cut-off	1	4	3	8	5	6
# false positives at that cut-off	2	3	13	0	15	14
# false negatives at 500 ng/mL	2	4	3	19	11	7
# false positives at 500 ng/mL	2	15	18	0	11	14

Discussion and conclusion

The best results were seen with the Abbott ADx assay that is not available anymore in Europe. If the cut-off is increased to 677 ng/mL, the AxSYM gives a low number of false positives and negatives. The new EMIT assay has excellent specificity, but misses more true positive samples: 2 samples containing amphetamine (225 and 253 ng/mL), 1 sample containing MDA (231 ng/mL), 4 samples containing MDMA (319-2760 ng/mL and MDA (113-516 ng/mL) and one sample containing amphetamine and MDMA). For the older EMIT assays and both KIMS methods, there was more overlap between negative and positive samples, resulting in a high number of false positives. The optimal cut-offs, calculated by analysis of the receiver operating characteristic curves, varied between 271 and 677 ng/mL. Use of 500 ng/mL cut-off doesn't change much for the ADx and KIMS X assays, increases the number of false positives for AxSYM and EMIT, and increases the number of false negatives for the new EMIT method and the KIMS method.

Keywords: amphetamine, MDMA, immunoassay