Pitfalls of LSD screening assays: comparison of KIMS and CEDIA immunoassays with LC-MS

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Pitfalls of LSD screening assays: comparison of KIMS and CEDIA immunoassays with LC-MS

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To the Editor,

Urine drug testing is widely applied in health care as well as forensic situations or even at the workplace. The simplicity of use and fast availability of results have increased the application of immunoassays to urine drug testing. However, these immunoassays are not impeccable and carry the risk of providing false-positive and false-negative results (1). Therefore, the results of immunoassays are considered only as presumptive until confirmed by a specific method like GC-MS or LC-MS. The effective screening assay should be characterized by high specificity for the target compound and sufficient sensitivity to detect drug or its metabolites at relevant concentrations.

Lysergic acid diethylamide (LSD) is one of the most potent hallucinogens known to man. However, its detection in urine is challenging due the very low doses ingested (moderate dose of 75-150 μg) and the rapid and extensive metabolism with the elimination half-life of 3-4 hours (2).

Several immunoassays for the LSD urine screening are commercially available for a variety of autoanalyzers. Depending on the affinity of antibodies and the detection technique, different assays may lead to the different screening results.

Under approval of the Cantonal Ethic Committee Zurich (KEK-ZH-Nr. 2015-0483), we conducted a retrospective analysis to confirm our LSD screening results in urine samples. Results obtained with our currently employed screening test, namely with the kinetic interaction of microparticles in solution (KIMS) assay from Roche Diagnostics performed on Cobas Integra 800 analyzer, were compared with those from the cloned enzyme donor immunoassay (CEDIA) from Microgenics performed on Cobas c502 analyzer. Afterwards, results of immunoassays were confirmed by LC-MS analysis.

In total, we analysed 50 urine samples as specified: KIMS screening on the fresh urine samples, followed by the replicated KIMS and CEDIA assays and LC-MS analysis on the

frozen urine samples (Table 1). The KIMS assay was replicated to ensure that preanalytical conditions are uniform for all three measurements.

Our first rather unanticipated finding was a high number of the discrepant results between two KIMS assays: 14 urine samples were positive for LSD in the first screening but negative in the replicated assay and four urine samples were primarily negative but positive in the succeeding analysis (Table 1). Altogether, 36% of the results were discrepant (Figure 1A). Since there is no significant loss of LSD in the frozen state or after several freeze/thaw cycles (3, 4), this observed discrepancy cannot be explained only by different preanalytical conditions of the urine samples. For the KIMS immunoassay, triplicate measurement of the 0.5 ng/ml LSD cut-off calibrator is used a reference to distinguish between positive and negative results. According to the test principle, the absorbance reduces proportionally to the increasing LSD concentration in the sample. Thus, the positive control containing 1 ng/ml LSD and the negative control containing 0.25 ng/ml LSD will produce signals below and above the cut-off calibrator, respectively. At higher absorbance values of the cut-off calibrator, negative controls may falsely fall below it, therefore being classified as positive. This problem was frequently observed in our laboratory and a new calibration to obtain lower absorbance values of the cut-off calibrator was required in order to pass the quality control. As suggested by our results, the same could apply to the urine samples.

When compared with the LC-MS, KIMS assay showed rather poor total concordance of 63.5% (Figure 1B). On the one hand, this might be attributed to poor analytical quality of the KIMS immunoassay as described above. On the other hand, it may be caused by interfering substances present in the urine. However, a general unknown screening with LC-MS did not identify any interfering substance.

The CEDIA immunoassay allows both qualitative and semiquantitative applications. In the qualitative application only one 0.5 ng/ml cut-off calibrator is used as the reference. In our laboratory we apply semi-quantification and use one calibrator without LSD (negative

calibrator), and three calibrators containing LSD at the cut-off (0.5 ng/ml), with intermediate (1.5 ng/ml) and high (3.0 ng/ml) LSD concentration. The CEDIA assay showed overall satisfactory total concordance of 92.3% with LC-MS (Figure 1C). Two urine samples, which were positive for LSD in the CEDIA assay were negative in LC-MS, contained fentanyl. The cross-reactivity of fentanyl with the CEDIA assay is already known to cause false-positive findings (5).

Furthermore, the comparison of the results from both immunoassays revealed an unacceptable high percentage (36.5%) of discrepant results (Figure 1D).

It is worth to mention that two urine samples were positive in the confirmatory LC-MS analysis, but negative when analyzed with either immunoassay (Table 1). Except for very low creatinine concentration (1.0 and 1.9 mmol/l) indicating strong urine dilution, urine samples were not adulterated with glutaraldehyde, bleach, or pyridinium chlorochromate as shown by Intect 7 Urine Adulteration Test Strip. We speculate that discrepancies between immunoassays and LC-MS can be caused by other adulterant(s), lower sensitivity of immunoassays or urine dilution.

To sum up, LSD screening using immunoassays have to be interpreted with the knowledge of the limitations of each assay. Moreover, as a rule, the results of drug screening in the urine using immunoassays should be considered as “presumptive positive” until confirmed by the MS-based technique.

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Tabele 1

Results of KIMS and CEDIA assays and confirmatory LC-MS in 52 analysed urine samples.

[*]fresh urine samples, [#] urine samples after 1 freeze-thaw cycle.

Nr. of urine samples	first KIMS screening[*]	replicated KIMS assay[#]	CEDIA assay[#]	LC-MS[#]
2	positive	positive	positive	detectable
13	positive	positive	negative	undetectable
14	positive	negative	negative	undetectable
4	negative	positive	negative	undetectable
2	negative	negative	positive	undetectable
15	negative	negative	negative	undetectable
2	-	negative	negative	detectable

Figure 1

Concordance of obtained results between first KIMS screening and replicated KIMS assay (A), replicated KIMS assay and MS (B), CEDIA assay and MS (C), and replicated KIMS and CEDIA assays (D).