Screening oral fluids for cocaine and cannabinoids by ELISA: development of analytical procedures and implementation in a field trial on 2570 truck drivers


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Introduction

There is growing interest in the estimation of impairment in operators of motor vehicles by drugs of abuse. Using oral fluid to assess impairment is an ultimate objective. This study describes the development of screening procedures for drugs of abuse in oral fluid (saliva) by ELISA. These methods were applied to a project led by the SAAQ on 2570 truck drivers in the summer of 2001.

Methods

In this study 2570 truck drivers contributed 2177 urine and 2342 saliva samples. In 2006 instances, urine and saliva were matched. Saliva was sampled in Salivette oral fluid collection device (Sarstedt) immediately centrifuged upon reception in the laboratory and frozen at -20 °C.

Materials

Urine specimens were screened for drugs of abuse by ADX (immunofluorescence) using cutoffs of 300 ng/ml cocaine and 50 ng/ml cannabinoids. The presence of benzoylecgonine (cocaine metabolite) and carboxy-THC (cannabinoid metabolite) was confirmed by GC/MS.

Immunoassays

In saliva, screening methods were developed by ELISA using 96 well plates and commercially available kits for cocaine (Diagnostix Ltd., Mississauga, Ontario) and cannabinoids (Immunalysis, San Dimas, Ca), and run simultaneously on a P-lab robot (Biochem immunosystems).

For cocaine, 50 µL of saliva were dispensed into the 96 well plates and allowed to preincubate at 30 °C for 15 minutes to improve sensitivity. Cocaine conjugate was then added (150 µL) and competitive binding allowed to take place, incubating at 30 °C for one hour. The plate was then washed in phosphate buffer to remove interferences, 150 µL of enzyme substrate added, and the
plate incubated again at 30 °C for 15 minutes, to allow the enzyme reaction to convert substrate to product. After addition of 150 µL stop solution (1N HCl) absorbances were read at 450 nm.

The readings of absorbance of the reaction product are expressed in terms of inhibition of the blank (A/Ao x 100). The enzyme immunoassay was calibrated with benzylecgonine, the cutoff set at 2.5 ng/ml (52 % inhibition). A control material at a level of 5 ng/ml was prepared in saliva, and measured at every 10 positions on the 96 well plate. The same control sample was used throughout the project. Cross-reactivities were 100 % for benzylecgonine and 54 % for cocaine. Samples producing an inhibition less than that of the cutoff were reported as positive (presumptive).

For cannabinoids the objective was to optimise response towards Δ⁹THC. Enzyme immunoassays usually target the metabolite (carboxy-THC). The ELISA method proceeded as for cocaine using 96 well plates coated with an antibody specific for carboxy-THC and a nominal cross-reactivity of 60 % for Δ⁹THC. 50 µL of oral fluid, 100 µL of enzyme conjugate, 100 µL of substrate and 100 µL of stop solution were used. The enzyme immunoassay was calibrated with carboxy-THC because this substance remained much more stable in saliva than the native Δ⁹THC. A cutoff of 0.5 ng/ml carboxy-THC resulted in 36.7 % inhibition. A control material at a level of 10 ng/ml Δ⁹THC was prepared into fresh saliva daily, and measured at every ten positions of the 96 well plate. Actual cross-reactivity for Δ⁹THC was computed at 20 %. Samples producing an inhibition of less than 50 % of the blank were reported as positive (presumptive). Screening the 2342 saliva samples required 33 individual runs on 21 analytical days.

Confirmation by mass spectrometry

Saliva samples positive for cocaine were quantitated for both cocaine and benzylecgonine by tandem HPLC/MS/MS. 100 µL of saliva was used. The response was linear at levels between 0.1 and 1000 ng/ml of each substance, with a CV of 5 %.

Saliva samples positive for Δ⁹THC were quantitated by GC/MS according to the procedure published by D’Asaro. 500 µL saliva was used. The method was linear at levels between 2 and 50 ng/ml of Δ⁹THC, with a CV of 5 %.

Results

Statistics

a) Cocaine

In urine, 29 samples were confirmed positive (1.3 % of all 2177 urine). Only 1 urine was a false positive by immunoassay at the cutoff of 300 ng/ml.

In saliva, 37 samples were confirmed positive (1.6 % of all 2342 saliva). 67 samples were screened positive, 65 were available in sufficient volume to allow confirmation.
In 2006 cases where matched urine and saliva were available, 23 urine samples were positive for cocaine metabolite and 17 matching saliva samples were also positive. Among positive urine samples, benzoylecgonine averaged 65.6 ng/ml and ranged between 0.5 and 547.8 ng/ml. Among positive saliva samples cocaine and benzoylecgonine were in the range 0.4 to 801.2 ng/ml, and 0.6 to 763.7 ng/ml, averaging 39.5 ng/ml and 70.1 ng/ml.

b) $\Delta^9$THC

In urine, 103 samples were confirmed positive (4.7 %). In all 1 urine was a false positive by immunoassay at the cutoff of 50 ng/ml.

In saliva, 14 samples were confirmed positive (0.6 %). 137 samples were screened positive by ELISA, but sufficient volume was available for confirmation in only 73 instances.

In 2006 matched urine and saliva, 83 urine were positive for carboxy-THC and only 9 matching saliva samples were positive for $\Delta^9$THC. No carboxy-THC was found in any saliva. In 9 instances, the volume of matching saliva was insufficient to complete the comparison. In all positive urine samples, carboxy-THC averaged 442.2 ng/ml and ranged between 16.2 ng/ml and 3925.4 ng/ml. In all positive saliva samples, $\Delta^9$THC averaged 12.3 ng/ml and ranged between 2.2 and 51.9 ng/ml.

Methodologies

ELISA screening

ELISA screening was achieved in 33 analytical runs in 21 calendar days. For cocaine, the cutoff read on average 52.3 % inhibition, and the 5 ng/ml control sample 16 % inhibition (CV = 6%). For $\Delta^9$THC the cutoff read on average 36.7 % inhibition and the 10 ng/ml control sample 16.6 % inhibition (CV = 15%).

Stability of $\Delta^9$THC in saliva and aqueous standards was problematic. Standards of carboxy-THC and $\Delta^9$THC in methanol were stable for over two years. But, the same species, while in aqueous solutions, were stable for less than a month for the first and no more than a few days for $\Delta^9$THC at levels below 100 ng/ml. In the GC/MS confirmation, adsorption of $\Delta^9$THC onto surfaces of plastic and glass was a problem.

Volume of collected oral fluid

Saliva collection devices were found empty after centrifugation on 204 instances among 2546 (8.0%) and sufficient volume was available for screening in 2342 devices. A properly collected Salivette usually produces more than 2 ml of oral fluid, but this was rarely the case in this survey.

Conclusion

The data gathered here show that, for cocaine at least, the saliva level is a very good indicator of exposure. We have found that, under the conditions of collection used in this survey, the
concentrations of cocaine and its metabolite benzoylecgonine are just as elevated in saliva as they are in urine; we have not seen comparable data published elsewhere.

The frequency of positive saliva specimen was also close to that of urine samples. The sensitivity of the ELISA procedure is completely adequate.

On the contrary, screening and confirmation of $\Delta^9$THC in saliva is difficult. The levels of $\Delta^9$THC are low, the stability of $\Delta^9$THC standards is not assured, and the selectivity of antibody material does not favour the binding of this species. The impact on the stability of collected saliva is unknown.

At present, there is no way to measure efficiency of the collection device in terms of percentage analyte recovered (or expected concentration). Many questions surrounding pharmacological issues need to be investigated and can only be answered through studies involving human volunteers. Interindividual variations in saliva transfer of drugs, relationship between levels in saliva and actual impairment, delay of detection, speciation, all remain to be studied.

Reference: