Short Communication

The Prevalence of Alcohol and Drugs in Sampled Oral Fluid is Related to Sample Volume

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Abstract

About 22,000 samples of oral fluid have been collected in five population studies in Norway using either the Intercept® or Statsure™ oral fluid sampling devices. The prevalence of alcohol and drugs was found to be higher in oral fluid samples with small volumes than in those with large volumes for both sampling devices; the largest differences were observed for tetrahydrocannabinol, alcohol, amphetamines, and cocaine/benzoylecgonine when using the Statsure device. Our recommendation is that samples of oral fluid with smaller volume than required by the analytical methods should not be discarded, but instead be analyzed using a smaller sample volume, if necessary, after dilution. If not analyzed, positive drug cases will be missed, and the total prevalence of alcohol and drugs in the population being studied will be underestimated.

Introduction

A number of medicinal drugs (1,2) and illegal drugs (3–6) may reduce salivary flow and cause short-term hyposalivation or chronic xerostomia. In these cases, the volume of oral fluid collected using a commercially available sampling device may be small and in some cases less than the volume required by the analytical methods.

A roadside survey of alcohol, drugs, and driving was performed in Norway in 2005–2006 using the Intercept® Oral Specimen Sampling Device (Orasure Technologies, Bethlehem, PA) to collect samples of oral fluid (7). A second study was performed 2008–2009 as part of the European DRUID project using Statsure Saliva Sampler™ (Saliva Diagnostic Systems, Framingham, MA). In addition, we have performed three other population studies using oral fluid collected with the Statsure device during 2008–2010. Drug findings for different oral fluid sample volume intervals are presented in this report.

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Materials and Methods

Samples of oral fluid were collected using the Intercept Oral Specimen Collection Device or the Statsure Saliva Sampler. The collection time for the Intercept device was 2 min, and for the Statsure device, collection was performed until the volume indicator turned blue or for a maximum of 5 min if the indicator did not turn blue. The Intercept and Statsure devices contained 0.8 and 1.0 mL buffer, respectively. The collected volumes of oral fluid were determined for each sample by weighing.

Alcohol was determined by an automated enzymatic method (8) using 100 µL oral fluid/buffer mixture. Drugs were extracted from 500 µL oral fluid/buffer mixture using liquid–liquid extraction and analyzed with liquid chromatography with tandem mass spectroscopic detection (9). In cases where less than 500 µL oral fluid/buffer mixture was available for analytical testing, smaller volumes were extracted with the same amount of solvent after adding the regular volume of internal standard solution and buffer, as stated in the analytical method. Samples with volumes less than 100 µL were not analyzed; these were regarded as failed oral fluid sample collections.

The dilution of native oral fluid was expected to be different for the two sampling devices. For the Intercept device, the manufacturer stated that an average of 0.4 mL oral fluid would be collected, which would be diluted with 0.8 mL buffer present in the sampling device. For the Statsure device, an average of 1.0 mL oral fluid was expected to be collected, which would be diluted with 1.0 mL buffer. Different analytical cut-off thresholds were therefore used for the two devices in an attempt to obtain comparable results. However, for alcohol and some drugs we needed to use lower cut-off values to comply with requirements set by the DRUID project. All analytical cut-off thresholds were equal to or larger than the quantification limits of the analytical methods. The same cut-off thresholds were used for high-volume and low-volume samples.

Reporting thresholds for drug concentrations in undiluted oral fluid were set based on a combination of analytical capa-

bilities and expected pharmacologically relevant thresholds (7). Analytical cut-off and reporting thresholds are presented in Table I.

Pearson's two-sided chi square test for categorical data was calculated using SPSS (version 14.0, Chicago, IL) and used for the statistical evaluation of findings.

Table I. Analytical Cut-Off and Reporting Thresholds

Substance	Analytical Cut-Off Thresholds for Oral Fluid/Buffer Mixtures (ng/mL)		Reporting Thresholds for Undiluted Oral Fluid (ng/mL)
	Intercept	Statsure	
Alcohol	0.05 mg/mL	0.05 mg/mL	0.1 mg/mL
Alprazolam	0.15	0.23	1
Amphetamine	9.1	12.2	25
Benzoylecgonine	2.4	3.6	10
Carisoprodol	8.6	13.0	50
Clonazepam	0.16	0.24	0.5
Cocaine	0.61	0.91	10
Codeine	2.5	3.7	20
Diazepam	0.24	0.18	1
Flunitrazepam	0.10	0.16	0.3
Lorazepam	0.55	0.48	1.6
Meprobamate	7.2	10.9	1000
Methadone	2.6	3.9	20
Methamphetamine	4.9	7.5	25
Morphine	2.4	3.6	10
Nitrazepam	0.14	0.21	0.5
Nordiazepam	0.22	0.34	1
Oxazepam	1.4	2.4	5
Tetrahydrocannabinol (THC)	0.26	0.31	1
Zolpidem	0.05	0.08	10
Zopiclone	0.32	0.49	10
3,4-Methylenedioxyamphetamine (MDA)	Not analyzed	9.0	25
3,4-Methylenedioxy-*N*-ethylamphetamine (MDEA)	6.8	10.3	25
3,4-Methylenedioxy-*N*-methylamphetamine (MDMA)	1.6	1.9	25
6-Monoacetylmorphine	0.27	0.41	5

Table II. Prevalence (%) of Alcohol and Drugs Within Different Volume Intervals of Oral Fluid Collected with the Intercept Oral Specimen Collection Device

Oral Fluid Volume	< 0.20 mL	0.20–0.29 mL	0.30–0.39 mL	0.40–0.59 mL	≥ 0.60 mL	p
No. of samples	2451	2700	1779	2098	1788	—
Alcohol or drugs	6.3	4.8	4.2	3.5	3.4	< 0.001
Alcohol	0.7	0.4	0.1	0.3	0.2	0.005
Medicinal drugs*	4.2	3.3	3.5	2.9	2.8	0.067
Zopiclone	2.0	1.4	1.7	1.1	1.3	0.173
Diazepam or nordiazepam	1.1	0.7	0.8	0.7	0.8	0.424
Illegal drugs†	1.9	1.1	0.8	0.4	0.5	< 0.001
Amphetamines	0.4	0.3	0.2	0.1	0.2	0.347
Cocaine or benzoylecgonine	0.1	0.2	0.1	0.1	0.1	0.551
THC	1.5	0.6	0.6	0.1	0.2	< 0.001

* Includes alprazolam, carisoprodol, clonazepam, codeine, diazepam, flunitrazepam, lorazepam, meprobamate, methadone, morphine, nitrazepam, nordiazepam, oxazepam, zolpidem, and zopiclone.
† Includes amphetamine, benzoylecgonine, cocaine, methamphetamine, THC, MDA, MDEA, MDMA, and 6-monoacetylmorphine.

Table III. Prevalence (%) of Alcohol and Drugs Within Different Volume Intervals of Oral Fluid Collected with the Statsure Saliva Sampler

Oral Fluid Volume	< 0.50 mL	0.50–0.74 mL	0.75–0.99 mL	1.00–1.24 mL	≥ 1.25 mL	*p*
No. of samples	1202	1122	4069	3999	536	–
Alcohol or drugs	18.1	6.4	4.4	2.7	3.0	< 0.001
Alcohol	4.5	0.9	0.3	0.3	0.4	< 0.001
Medicinal drugs	9.3	3.7	3.1	2.0	2.4	< 0.001
Zopiclone	3.0	1.4	1.2	0.7	1.1	< 0.001
Diazepam or nordiazepam	3.9	1.2	1.0	0.8	0.7	< 0.001
Illegal drugs	9.7	2.9	1.5	0.7	0.6	< 0.001
Amphetamines	4.2	0.8	0.5	0.2	0.4	< 0.001
Cocaine or benzoylecgonine	2.3	0.5	0.2	0.2	0.0	< 0.001
THC	6.5	2.7	1.1	0.4	0.2	< 0.001

Table IV. Average Volumes (mL) of Oral Fluid Collected in Relation to Sampling Device and Substance Findings

Substance	Intercept Volume (mL)	Intercept *n*	Statsure Volume (mL)	Statsure *n*
No substance found	0.35	10,302	0.91	10,355
Alcohol or drugs	0.30	492	0.67	592
Alcohol	0.25	38	0.44	87
Medicinal drugs	0.32	365	0.74	365
Zopiclone	0.31	163	0.75	134
Diazepam or nordiazepam	0.32	87	0.71	133
Illegal drugs	0.26	110	0.58	240
Amphetamines	0.31	31	0.52	86
Cocaine or benzoylecgonine	0.34	14	0.55	50
THC	0.21	69	0.57	168

Results and Discussion

A total of 10,816 samples of oral fluid samples collected with the Intercept device were analyzed. These samples contained collected oral fluid mixed with 0.8 mL buffer present in the sampling device. The oral fluid/buffer mixture was recovered from the device by centrifugation of the sample tube. Practically all the oral fluid/buffer mixture was recovered and available for analytical testing. The average volume of collected oral fluid was 0.34 mL. If only 0.2 mL oral fluid was collected, 1.0 mL oral fluid/buffer mixture was available for analytical testing. Results for alcohol, medicinal drugs, illegal drugs, and some of the most prevalent drugs or drug groups in undiluted oral fluid were calculated, and findings in relation to original sample volume are presented in Table II.

A total of 10,928 samples of oral fluid collected with the Statsure device were analyzed. These samples contained collected oral fluid mixed with 1.0 mL buffer. Before analytical testing, the sampling pad was disconnected from the sampling pad stem and placed into the sample tube, and a filter tube was inserted into the sample tube to recover the oral fluid/buffer mixture. However, about 1.0 mL oral fluid/buffer mixture could not be recovered and remained in the sample tube absorbed by the sampling pad. Thus, if 0.2 mL oral fluid was collected, only about 0.2 mL oral fluid/buffer mixture was recovered from the sampling device. Therefore, either alcohol or drugs were analyzed in samples with volumes between 0.1 and 0.2 mL oral fluid/buffer mixture. The average volume of collected oral fluid was 0.90 mL when using the Statsure device. Analytical results are presented in Table III.

For both sampling devices, higher prevalences of alcohol and drugs were found among oral fluid samples with small volumes. The prevalence decreased with increasing sample volume but was statistically significant for a larger number of substances when using the Statsure sampling device than when using the Intercept device. Particularly large differences between small- and large-volume samples were observed for tetrahydrocannabinol (THC), alcohol, amphetamines, and cocaine/benzoylecgonine using the Statsure device. Less marked differences were observed for medicinal drugs.

The average volume of collected oral fluid in relation to substance findings are presented in Table IV. The average volumes of oral fluid were lower in samples where alcohol or some drugs were found, especially for the Statsure device.

The Intercept sampling pad is made of cotton treated with a solution containing sodium chloride, citric acid, sodium benzoate, potassium sorbate, gelatine, and sodium hydroxide, according to the package insert; it thus contains chemicals that stimulate the production of oral fluid. The Statsure sampling pad is made with cellulose and is not treated with any chemicals stimulating the production of saliva does not (10). Chemical stimulation of oral fluid production did not eliminate the higher prevalence of drugs and alcohol in small-volume sam-

ples; however, the difference between small- and large-volume samples was smaller.

Our findings suggest that oral fluid samples with volume less than normally required by the analytical methods should not be excluded from alcohol and drug analysis. In cases where small volumes of oral fluid/buffer mixture are available, samples should be analyzed, if necessary, after diluting the samples, and smaller samples volumes or dilutions should be corrected for in later calculations. This recommendation applies when using the Statsure device because the Intercept device does not have the same problem of retained liquid that is not available for analytical testing.

In some cases with small volume of oral fluid, the dilution with buffer may cause drug concentrations below the analytical cut-off, even if the concentration in native oral fluid is high. However, we experienced that the drug concentrations in many of the small-volume samples were high in spite of the large dilution with buffer. This was, in many cases, due to the use of large doses of drugs that are known to cause hyposalivation.

Acknowledgments

References

1. A. Wolff, L. Zuk-Paz, and I. Kaplan. Major salivary gland output differs between users and non-users of specific medication categories. *Gerodontology* **25:** 210–216 (2008).
2. C. Scully. Drug effects on salivary glands: dry mouth. *Oral Dis.* **9:** 165–176 (2003).
3. C. McGrath and B. Chan. Oral health sensations associated with illicit drug abuse. *Br. Dent. J.* **198:** 159–162 (2005).
4. D.T. Hamamoto and N.L. Rhodus. Methamphetamine abuse and dentistry. *Oral Dis.* **15:** 27–37 (2009).
5. P.A. Versteeg, D.E. Slot, U. van der Velden, and G.A. van der Weijden. Effect of cannabis usage on the oral environment: a review. *Int. J. Dent. Hyg.* **6:** 315–320 (2008).
6. E.C. Donny, G.E. Bigelow, and S.L. Walsh. Assessing the initiation of cocaine self-administration in humans during abstinence: effects of dose, alternative reinforcement, and priming. *Psychopharmacology (Berl.)* **172:** 316–323 (2004).
7. H. Gjerde, P.T. Normann, B.S. Pettersen, T. Assum, M. Aldrin, U. Johansen, L. Kristoffersen, E.L. Øiestad, A.S. Christophersen, and J. Mørland. Prevalence of alcohol and drugs among Norwegian motor vehicle drivers: a roadside survey. *Accid. Anal. Prev.* **40:** 1765–1772 (2008).
8. L. Kristoffersen and A. Smith-Kielland. An automated alcohol dehydrogenase method for ethanol quantification in urine and whole blood. *J. Anal. Toxicol.* **29:** 387–389 (2005).
9. E.L. Øiestad, U. Johansen, and A.S. Christophersen. Drug screening of preserved oral fluid by liquid chromatography–tandem mass spectrometry. *Clin. Chem.* **53:** 300–309 (2007).
10. K. Langel, C. Engblom, A. Pehrsson, T. Gunnar, K. Ariniemi, and P. Lillsunde. Drug testing in oral fluid-evaluation of sample collection devices. *J. Anal. Toxicol.* **32:** 393–401 (2008).