



Oral fluid drug tests: Effects of adulterants and foodstuffs

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Abstract

An on-site oral fluid drug screen, Oratect[®], was used to investigate the effects of adulterants and foodstuffs on oral fluid test results. Common foods, beverages, food ingredients, cosmetics and hygienic products were demonstrated not to cause false positive results when tested 30 min after their consumption. Evaluations of two commercial oral fluid adulterants, “Clear Choice[®] Fizzy Flush[™]” and “Test’in[™] Spit n Kleen Mouthwash” suggest their mechanism of action is the clearing of residual drugs of abuse compounds through rinsing of the oral cavity. They do not directly destroy the drug compounds or change the pH of the oral fluid. It is also suggested that a common mouthwash would perform similar action.

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1. Introduction

To cover up the use of illicit drugs, users have tried many different ways to defeat drug detection. A published report from Quest Diagnostics [1] indicated that the detectable adulteration rate in the U.S. ranges from 2.67% of all positive urine samples in year 1999 to 1.10% in year 2003. Common means of urine adulteration include substitution, dilution and addition of chemicals [2]. To counter this trend, regulatory guidelines have been established in the U.S. for direct observed specimen collection and adulteration testing [3].

As an alternative specimen for drugs of abuse (DOA) testing, oral fluids appear to reduce the susceptibility to adulteration due to easy observation of the sample collection. However, with the introduction of on-site saliva DOA tests, saliva adulteration reagents are beginning to appear for sale on the Internet.

In this report, the performance of an on-site oral fluid test device and its interactions with normal materials frequently

found in oral fluids were first studied. This device was then used to monitor oral DOA concentrations in volunteers after smoking a marijuana cigarette, eating poppy seed muffins and drinking a dose of codeine containing cough medicine. Finally, the effects on the oral fluid drug tests of two commercially available adulterants and a mouthwash were evaluated.

2. Materials and methods

2.1. Specimens

Oral fluids were obtained from volunteers by spitting into small cups. The fluids were pooled and centrifuged to remove insoluble particles. A sample of processed pooled oral fluid was analyzed with GC/MS to ensure that it was drug free. The pooled oral fluid was spiked with various concentrations of DOA and then used to validate the cut-off values for all tests on the test device. In addition, positive and negative samples were obtained from consenting volunteers in a rehabilitation center.

Positive urine controls containing 150 ng/ml of 11-nor-9-carboxy- Δ^9 -tetrahydrocannabinol (THC-COOH) and

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6000 ng/ml of morphine were purchased from Biochemical Diagnostics, Inc. (Edgewood, NY, USA).

2.2. Test devices

For oral fluid DOA determination, an on-site drug screen named Oratect[®] manufactured by Branan Medical Corporation was used. It utilizes a colloidal gold particle based lateral flow immunoassay and combines sample collection and drug testing in a single device [4]. By inserting the device into a volunteer's mouth, oral fluids flowing to a collection pad would be saturated after about 1–3 min. The fluids would then migrate by capillary action to a conjugate pad and re-dissolve the drug-antibody-gold conjugates on the pad. Subsequently, the fluid would carry the gold conjugate across a nitrocellulose membrane immobilized with drug-protein conjugates in a line form. Finally, all the liquid components would be absorbed by an absorption pad on the other end of the device.

In the absence of drug, the drug-antibody-gold conjugate would react with individually immobilized drug-protein lines on the membrane to form a clear red line. When a specific drug is present in the oral fluid sample above the cut-off concentration, it will saturate the antibody binding sites on the drug-antibody-gold conjugate and prevent the formation of a red line and thus provide a positive result.

For confirmation of positive results, the collection pads were detached, stored in a vial containing 0.8 ml of phosphate buffer solution and sent to Scientific Testing Laboratories, Inc. (Richmond, VA, USA) for GC/MS analysis.

The cut-off DOA concentrations of the Oratect device and the laboratory GC/MS procedure are shown in Table 1.

The Intercept[®] device from Orasure Technologies, Inc. (Bethlehem, PA, USA) was used as a reference method. Oral fluids were collected with the Intercept devices according to the product insert and sent to Scientific Testing Laboratories, Inc. for quantitative analysis by GC/MS.

Monitect[®] PC-11 5-panel urine drug screens from Branan Medical Corporation were utilized for the qualitative assay of DOA in urine samples.

Table 1
Cut-off DOA concentrations for Oratect and GC/MS procedure

Drug	Cut-off values for GC/MS	Cut-off values for Oratect
Cocaine	4	20
<i>d</i> -Methamphetamine	16	50
<i>d</i> -Amphetamine	16	50
Opiates	4	20
Phencyclidine (PCP)	1	4
Δ^9 -Tetrahydrocannabinol (THC)	0.2	40

Numbers in ng/ml.

2.3. Adulterants

Two commercial adulterants purchased via the Internet were investigated. In addition, a common mouthwash was evaluated for its potential use as adulterant. Both commercial adulterant products have money back guarantees on the label.

Adulterant A was “Clear Choice[®] Fizzy Flush[™]” manufactured by Health Tech (Atlanta, GA, USA). Its label listed components as potassium, sodium, creatine monohydrate, vitamin B2 and proprietary ingredients including Uva Ursi, dandelion, cranberry, ginkgo biloba and stevia. Each box of this product contained two 5 g effervescent tablets. To use, one tablet is dissolved in 300 ml of water. The directions for use suggest swishing the solution in the mouth for 10 s and then swallowing it. This process is to be repeated until the entire content has been consumed. The solution was yellowish brown in color, smelled like an orange juice drink, and tasted bitter sweet. Black particles were found settling on the bottom of the container after the tablet was dissolved.

Adulterant B was “Test'in[™] Spit n Kleen Mouthwash” manufactured by A-Z Enterprises (Sparks, NV, USA) which listed magnesium, glycerin, chloride, witch hazel, sodium, aloe vera, potassium, mint, ascorbic acid, sulfate, lithium and boron as ingredients. It comes in a vial containing 15 ml of liquid. The instruction for use is to rinse mouth and hold it for three minutes. This process is to be repeated several times. It also recommends the user to consume five breath fresheners (not supplied) before test. The solution from the vial was clear blue with a mint smell and had a very salty taste.

The mouthwash studied was “Cool Mint Listerine[®] Antiseptic Mouthwash” manufactured by Pfizer Consumer Healthcare, Morris Plains, NJ, USA. In the study, volunteers rinsed their mouths with 20 ml of Listerine for 20 s.

2.4. Method for validation of Oratect precision

The pooled negative oral fluids were spiked with 0.25, 0.5, 0.75 and 3 times the cut-off concentrations of cocaine, opiate, THC, *d*-amphetamine, *d*-methamphetamine and PCP. Twenty samples were run for each concentration.

2.5. Method for the study on interfering substances

In a food interference study, three groups of volunteers each consumed a full course of ethnical dish including Mexican beef burritos, Vietnamese Pho noodles, and American continental food of fry chicken or beef. After thirty minutes, they were self-tested with the Oratect device according to the product insert.

In another study, potential interfering food ingredients including sugar, table salt and sodium glutamate were added to pooled negative oral fluid sample to give final concentration of 2% sugar, 0.5% table salt and 0.1% sodium glutamate. 0.5 ml of each of these spiked samples was added drop

Table 2
Precision of the Oratect device

Drug	Drug concentration as % of cut-off				
	0%	25%	50%	75%	300%
Cocaine	20–	20–	20–	18–/2+	20+
Opiate	20–	20–	20–	18–/2+	20+
THC	20–	20–	20–	18–/2+	1–/19+
Amphetamine	20–	20–	20–	18–/2+	20+
Methamphetamine	20–	20–	20–	18–/2+	20+
PCP	20–	20–	20–	18–/2+	20+

“–” indicates negative result and “+” indicates positive result.

wise to the Oratect collection pad and tested for their effects on the test results. Similarly, specific beverages such as tea, black coffee, sodas and juices were added to negative oral fluid to obtain final concentrations of 10% and tested.

Moreover, 10 volunteers were each self tested with the Oratect device 30 min after they had brushed their teeth with Colgate Total[®] toothpaste (New York, NY, USA), rinsed their mouth with Listerine[®] mouthwash, applied various brands of lipsticks, chewed one stick of Wrigley[®] gum (Chicago, IL, USA) or smoked one cigarette (various brands).

It is believed that 30 min would be sufficient time for food particles and other materials to clear the oral cavity. It would also allow equilibration of oral fluid to occur so that accurate test results can be obtained.

2.6. Method for study of direct adulterant effects on DOA

Since the proposed mechanisms of action of some urine adulterants are through changing sample pH or destruction of the DOA molecules, the direct effects of the two commercial oral adulterants were evaluated with a readily available solution containing DOA such as a urine drug control. Each of the adulterant was spiked into a commercial positive urine control, containing 150 ng/ml of THC-COOH and 900 ng/ml of morphine, to a final concentration of 5% adulterant. Both the spiked control and untreated controls were incubated at room temperature for one hour. Then, the presence of THC-COOH and morphine in the controls were tested with the Monitect devices by adding them to the collection pads.

Table 3
Food interference study results

Food	Test results
Asian food (<i>N</i> = 10)	Negative
Hispanic food (<i>N</i> = 10)	Negative
American continental food (<i>N</i> = 10)	Negative
Sugar, 2%	Negative
Table salt, 0.5%	Negative
Sodium glutamate, 0.1%	Negative

Table 4
Beverage effect

Beverage	Test results
Lipton [®] Tea (dark)	Negative
Black Coffee	Negative
Pepsi Cola [®]	Negative
Dr. Pepper [®]	Negative
Diet Coca Cola [®]	Negative
Orange Juice	Negative
Apple Juice	Negative

In addition, the pH values of the original adulterant solutions were measured with a pH meter.

2.7. Method for evaluating the *in vivo* action of adulterants on THC

One hour after two volunteers had each smoked one marijuana cigarette, they were tested for THC using Oratect and Intercept devices. Within 10 min after these tests, the volunteers used one of the two adulterants according to its product instruction and were again tested with the two devices 10 min later. Both the Oratect and Intercept were sent for GC/MS analyses. On the next day, the experiment was repeated using the other adulterant.

2.8. Method for time study of THC

For comparison purpose, Oratect and Intercept tests were performed at 30 min intervals on three volunteers after each had smoked one marijuana cigarette and did not use the adulterants. In this experiment, 10 min after smoking the marijuana cigarette, they were tested for THC using Oratect and Intercept devices every 30 min for 2 h. The test results of Oratect were recorded on-site and the oral fluid samples in the specified collectors were sent out for GC/MS analysis.

2.9. Method for evaluating the *in vivo* action of Adulterant A and mouthwash on codeine

Twenty minutes after one volunteer took a single dose of 10 mg of codeine phosphate (Prometh with Codeine Cough Syrup manufactured by Alpharma USPD Inc., Baltimore, MD, USA), he was tested for opiates using Oratect and Intercept devices. Then, the volunteer used Adulterant A and

Table 5
Cosmetic and hygienic product effect on Oratect

Cosmetic and hygienic products	Test results
Colgate [®] Toothpaste	Negative
Listerine [®] Mouthwash	Negative
Lipstick (various brands)	Negative
Wrigley [®] Gum	Negative
Cigarette (various brands)	Negative

Table 6
Direct adulterant effect on DOA

	Test results	
	Adulterant A	Adulterant B
THC-COOH in untreated control	Positive	Positive
THC-COOH in control spiked with adulterant	Positive	Positive
Morphine in untreated control	Positive	Positive
Morphine in control spiked with adulterant	Positive	Positive

retested 10 min afterward. This procedure was repeated the next day substituting mouthwash for the Adulterant A. Both the Oratect and Intercept were sent for GC/MS analyses.

2.10. Method for evaluating the *in vivo* action of mouthwash on opiate after the consumption of a poppy seed muffin

Two volunteers each consumed one (180 g) poppy seed muffin (purchased from Costco Store, Irvine, CA, USA), were tested for opiate using Oratect and Intercept devices after 30 min. Then these volunteers rinsed with the mouthwash and were again tested for the opiate 30 min later. The Intercept devices were sent for GC/MS analysis.

3. Results and discussion

3.1. Validation of precision of the Oratect device

As shown in Table 2, the Oratect provided credible results for the food interference and adulterant studies.

3.2. Food interference on the Oratect device

After volunteers ate various ethnical foods, Oratect tests indicated negative results. Moreover, when specified food ingredients were spiked into negative oral fluid samples and tested with Oratect device they also showed negative results. Table 3 summarizes the test results of the food study. The test results suggest that normal food ingredients do not cause false positive results.

Table 7
In vivo adulterant effect on the THC

		THC results (ng/ml)			
		Adulterant A		Adulterant B	
		Subject 1	Subject 2	Subject 1	Subject 2
Before adulterant usage	Oratect	+	+	+	+
	Oratect GC/MS	66	88	64	77
	Intercept	71	96	72	82
After adulterant usage	Oratect	–	+	–	+
	Oratect GC/MS	25	44	22	48
	Intercept	31	45	14	44

“+” indicates positive result.

3.3. Effects of beverages

Pooled negative oral fluid samples spiked with 10% of the specified beverage solution all gave negative results with the Oratect device as shown in Table 4, suggesting that beverages commonly consumed do not cause false positive oral fluid test results.

3.4. Cosmetic and hygienic products

As shown in Table 5, various cosmetic and hygienic products did not give false positive results.

3.5. Direct effect of adulterant on DOA

To evaluate whether the commercial adulterants act by the destruction of DOA molecules, direct effect of these adulterants on a readily available solution containing DOA (urine drug controls) was studied. The results as shown in Table 6 suggest that these adulterants do not act by destruction of drug molecules as exemplified by THC-COOH and morphine. Moreover, pH determinations of the adulterant solutions showed that pH for Adulterant A and B was 6.5 and 7.2, respectively, suggesting that these adulterants do not act by drastic modification of pH.

3.6. In vivo effect of adulterants on THC

As shown in Table 7, there was a reduction of THC levels after the use of adulterants. However, it has been suggested that the presence of THC in oral fluids is due to the smoke residue of marijuana cigarette and that THC levels decrease

Table 8
Time course study of THC concentrations in oral fluids

	Subject A	Subject B	Subject C
0 h			
Oratect	–	–	–
0.5 h			
Oratect	+	+	+
Oratect GC/MS	494	512	393
Intercept	253	322	211
1 h			
Oratect	+	+	+
Oratect GC/MS	95	100	91
Intercept	NP	NP	NP
1.5 h			
Oratect	+	+	+
Oratect GC/MS	48	65	50
Intercept	50	72	46
2 h			
Oratect	±	+	±
Oratect GC/MS	34	44	33
Intercept	31	42	29
2.5 h			
Oratect	–	±	–
Oratect GC/MS	NP	25	NP
Intercept	NP	28	NP

“–” indicates negative result; “+” indicates positive result; “±” indicates a borderline result; NP indicates test not performed; in GC/MS and Intercept results are in ng/ml.

drastically over short period of time [5]. To demonstrate that the reduction of THC might be a normal phenomenon and not be due to the adulterant action, THC concentrations over the course of time were studied. The results as shown in Table 8 confirm that most of the reduction may be due to the natural decrease of THC concentration in oral fluids.

3.7. *In vivo* actions of Adulterant A and mouthwash on codeine

Similar to the THC effect, there was a reduction of opiate level in oral fluids after the usage of Adulterant A as shown in Table 9. However, such lowered level was still detectable

Table 9
Actions of Adulterant A and mouthwash on codeine

		Opiate results (ng/ml)	
		Adulterant A	Mouthwash
Before use	Oratect	+	+
	Oratect GC/MS	224	290
	Intercept	172	220
After use	Oratect	+	+
	Oratect GC/MS	68	87
	Intercept	58	81

Table 10
Effect of mouthwash on opiate readings

		Opiate results (ng/ml)	
		Subject 1	Subject 2
Before mouthwash use	Oratect	+	+
	Intercept	24	22
After mouthwash use	Oratect	–	–
	Intercept	<12	<12

“+” indicates positive result.

by Oratect. The decrease in opiate concentration might be due to the rinsing action and not due to any special formulation of the adulterant. Such suggestion was confirmed by the use of a regular mouthwash which was shown to give similar effect as Adulterant A.

3.8. *In vivo* action of mouthwash on opiate after the consumption of a poppy seed muffin

To support the theory that the adulterants act by clearing the residue of DOA by the rinsing action, volunteers tested for opiate after the consumption of a poppy seed muffin showed a decrease in oral fluid opiate concentration with the administration of mouthwash as shown in Table 10. Poppy seed muffin was used because much of the opiate found in oral fluids after muffin consumption should be due to poppy seed residue and not because of opiate metabolites. Hence, since mouthwash had similar effect to the oral opiate level, it can be suggested the adulterants in question probably act by rinsing action. Their clearing action is further enhanced by their high salt content.

4. Conclusions

We have evaluated two oral fluid adulterants and found that they are not effective in destroying DOA in solution. These adulterants act by cleansing the mouth and partially diluting the oral fluid sample. They are no more effective than a regular mouthwash in reducing the DOA level. In addition, self-testing by individuals using an oral fluid instant drug screen device named Oratect 30 min after the consumption of food, beverages, cosmetic and hygienic products were demonstrated to cause no false positive results.

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