An Evaluation of Nine Adulterants with the NeoSal® Oral Fluid Collection System and Neogen® ELISA Kits

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INTRODUCTION

One reason that oral fluid is an appealing matrix for drug testing is that samples are collected under direct observation[1,2]. While an observed collection may decrease the opportunity to tamper with a sample[3], it does not prevent individuals from trying to "cheat" the test with the use of an adulterant[2].

When these additives are successful, they are believed to work by diluting the sample, destroying or converting the drug or interfering with the test method[4]. This study focused on whether common oral fluid adulterants (and household items rumored to generate false negative results for drug of abuse testing) would interfere with the extraction efficiency of the NeoSal Oral Fluid Collection System or generate false negative results with Neogen Oral Fluid ELISA kits.

MATERIALS & METHODS

Samples and Adulterants

Three commercial adulterants and six common oral care items were purchased and used for this study. UltraKlean™ Ultra Wash, Supreme Klean Saliva Detox Mouthwash, Stinger Detox "Mouthwash" were found via internet search and each had been listed as generating a false negative in online discussion boards on this subject. These boards also listed common household oral care items as potential adulterants; Crest® Whitestrips®, Crest® Pro Health™ Listerine® Original Mouthwash (specifically, the original variety was mentioned as effective), Listerine® Pocketpaks® and Polident® Antibacterial Denture Cleanser (tablets) were selected for this study. A prescription mouthwash (active ingredient of chlorhexidine gluconate 0.12%) was also tested.

Evaluation of Neogen Oral Fluid ELISA Kits

Each liquid adulterant was diluted with NeoSal buffer at 5% v/v. The denture tablet, Whitestrip gel and Pocketpak strip were individually dissolved into DI water and subsequently added to the buffer. These samples were assayed in duplicate (along with a negative buffer control) across five oral fluid specific Neogen ELISA kits: Cocaine (Cat #120119), Methamphetamine (Cat #120219), Opiates (Cat #120319), PCP (Cat #120419) and THC (Cat #120519). A second set of adulterated buffer samples was spiked at 30 ng/mL for Benzoylecgonine, 50 ng/mL for Methamphetamine, 60 ng/mL for Morphine, 6 ng/mL for PCP and 8 ng/mL for Δ9-THC and also tested across all five ELISA kits.

Evaluation of the NeoSal Oral Fluid Collection System

Oral fluid was collected from four donors who self-identified as drug-free and samples were screened prior to testing, for confirmation. Each donor produced between 25-30 mL of expectorated oral fluid. 2 mL was removed from each sample to be kept as controls. The remaining volume was separated into 10, 2 mL aliquots; 9 for adulterants and one to be spiked but kept adulterant free. Liquid adulterants were added to individual aliquots at 5% v/v. The denture tablet and Whitestrip gel were individually dissolved into DI water and subsequently added to the oral fluid. For two donors, the Pocketpak strip was dissolved directly into the 2 mL aliquot. The other two individuals put a Pocketpak strip in their mouth and after one minute, expectorated 2 mL of oral fluid.

After adulteration, samples were spiked at 15 ng/mL for Benzoylecgonine, 25 ng/mL for Methamphetamine, 30 ng/mL for Morphine, 3 ng/mL for PCP and 4 ng/mL for Δ9-THC. 1 mL of each aliquot was diluted with NeoSal buffer and assayed directly, in duplicate. The remaining 1 mL was used in a simulated collection using a NeoSal Oral Fluid Collection Device. Samples that were collected using the NeoSal were allowed to incubate at room temperature for 24 hours before assaying across all five kits, in duplicate.

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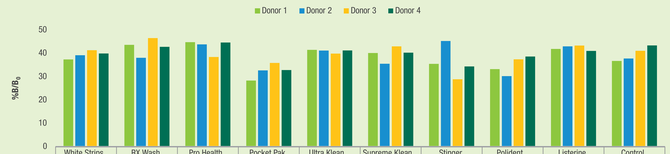
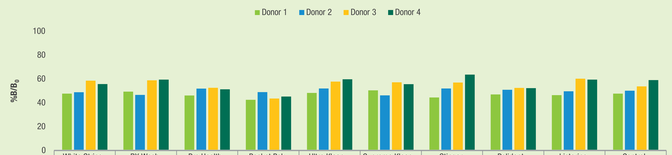
DATA

COCAINE KIT (BZE)



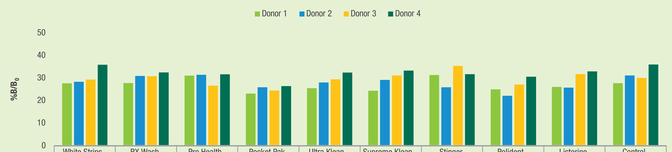
	White Strips	RX Wash	Pro Health	Pocket Pak	Ultra Klean	Supreme Klean	Stinger	Polident	Listerine	Control
Donor 1	37.3	43.6	44.7	28.3	41.4	40.1	35.4	33.2	41.8	36.7
Donor 2	39.1	38.0	43.8	32.6	41.1	35.5	45.2	30.2	42.9	37.7
Donor 3	41.3	46.5	38.4	35.8	39.8	42.9	28.8	37.4	43.3	41.0
Donor 4	39.9	42.7	44.6	32.8	41.2	40.2	34.3	38.6	41.0	43.3

METHAMPHETAMINE KIT (MAMP)



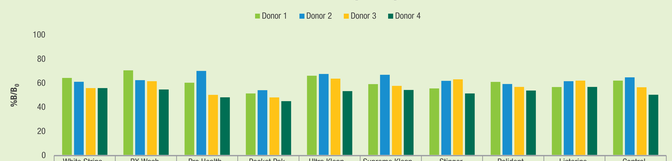
	White Strips	RX Wash	Pro Health	Pocket Pak	Ultra Klean	Supreme Klean	Stinger	Polident	Listerine	Control
Donor 1	47.8	49.4	46.2	42.6	48.4	50.6	44.5	47.1	46.5	47.8
Donor 2	49.0	46.7	52.0	49.1	52.1	46.3	52.1	51.0	49.8	50.3
Donor 3	58.6	59.0	52.7	43.7	57.9	57.3	57.1	52.6	60.4	53.8
Donor 4	55.8	59.6	51.4	45.4	59.9	55.7	63.7	52.4	59.5	59.2

OPIATES KIT (MORPHINE)



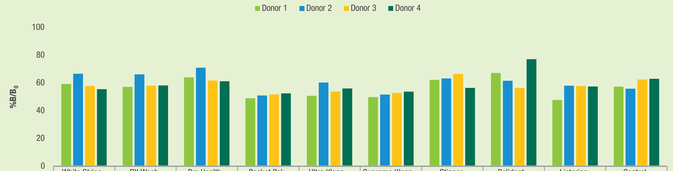
	White Strips	RX Wash	Pro Health	Pocket Pak	Ultra Klean	Supreme Klean	Stinger	Polident	Listerine	Control
Donor 1	27.6	27.7	31.1	23.1	25.5	24.3	31.3	25.0	26.1	27.7
Donor 2	28.3	30.9	31.4	25.9	28.0	29.1	25.9	22.1	25.7	31.1
Donor 3	29.3	30.8	26.6	24.4	29.4	31.1	35.4	27.1	31.7	30.0
Donor 4	35.9	32.4	31.6	26.4	32.4	33.3	31.7	30.6	32.9	36.0

PCP KIT (PCP)



	White Strips	RX Wash	Pro Health	Pocket Pak	Ultra Klean	Supreme Klean	Stinger	Polident	Listerine	Control
Donor 1	64.5	70.8	60.4	51.6	66.3	59.3	55.7	61.1	56.9	62.2
Donor 2	61.2	62.6	70.3	54.2	67.8	67.0	62.0	59.4	61.7	64.9
Donor 3	56.1	61.7	50.4	48.3	63.8	57.9	63.3	57.0	62.2	56.7
Donor 4	56.0	54.9	48.4	45.2	53.5	54.5	51.6	54.0	57.0	50.5

THC KIT (Δ9THC)



	White Strips	RX Wash	Pro Health	Pocket Pak	Ultra Klean	Supreme Klean	Stinger	Polident	Listerine	Control
Donor 1	59.3	57.2	64.0	49.0	50.7	49.8	62.3	67.2	47.7	57.3
Donor 2	66.6	66.2	71.0	51.0	60.3	51.6	63.3	61.5	58.1	55.9
Donor 3	57.8	58.0	61.7	51.7	53.8	52.9	66.5	56.5	57.8	62.5
Donor 4	55.5	58.2	61.2	52.5	56.0	53.7	56.4	77.1	57.4	63.0

RESULTS

Evaluation of Neogen Oral Fluid ELISA Kits

None of the adulterants displayed a significant increase in color development for any of the kits. All of the adulterated samples were compared to the negative buffer control and the variance among these samples is recorded below:

Kit	CV	SD
Cocaine/BZE	5.63%	0.07
Methamphetamine	5.20%	0.09
Opiates	4.78%	0.09
PCP	11.35%	0.10
THC	9.92%	0.16

The intraplate CV specification for Neogen kits is 10% and the results for the tested kits, except PCP, fell well below that number. The result for PCP was slightly over 10%; the Listerine Pocketpak sample showed lower color development than the other adulterated samples.

The spiked, adulterated buffer samples were compared to a multi-analyte control. All samples flagged as positive.

Evaluation of the NeoSal Oral Fluid Collection System

Absorbance values for adulterated samples were converted to $\%B/B_0$ values and compared to the unaltered control, spiked at the same drug concentrations. Six samples had results higher than 10% variance, with the highest being 15.08% less than the control.

CONCLUSIONS

None of the adulterants generated false negative results with any of the Neogen assays tested and it is unlikely that they interfere with ELISA testing methods.

The majority of the sample $\%B/B_0$ values were within 10% of the control and there were no obvious adulterant trends when comparing the results of the donors. While there may be some variability around the cutoff concentration, results indicate that there were no significant effect on the extraction efficiency of the NeoSal with the adulterated samples.

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