

# Considerations in The Use of Oral Fluid for Drug Testing

## SAMHSA – DTAB

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# Introduction – History of Oral Fluid Use

- Equine anti-doping analysis in the early 1900
- Used for clinical applications – advocated for DUI, workplace & etc.
- “Potential” OF-drug concentrations reflect blood-drug concentrations
- Collection advantages vs. blood or urine

# Nomenclature: Saliva/Oral Fluid(s)/Whole Saliva/Mixed Saliva & etc.

- Submandibular/ sublingual saliva: The fluid secreted mainly by the submandibular and **sublingual** glands and obtained from the floor of the mouth.
- The **parotid** gland is the 3<sup>rd</sup> major producer of saliva.
- **Minor salivary** glands (labial)
- Stimulated/Non-stimulated
- Figure 1
  - [http://www.google.com/imgres?imgurl=http://www.criminology.fsu.edu/journal/img/00003.gif&imgrefurl=http://www.criminology.fsu.edu/journal/hold.html&usg=\\_\\_u3c6ftQ5E5GS0kI3MrKhh9ltTM=&h=531&w=497&sz=11&hl=en&start=20&zoom=1&tbnid=pwVUM\\_wWgJCOsM:&tbnh=132&tbnw=124&ei=KfyATYjYBZLugQe7hdGnBw&prev=/images%3Fq%3Dhold%2B1976%2Bsaliva%2Bformation%26um%3D1%26hl%3Den%26safe%3Dactive%26sa%3DN%26tbm%3Disch&um=1&itbs=1](http://www.google.com/imgres?imgurl=http://www.criminology.fsu.edu/journal/img/00003.gif&imgrefurl=http://www.criminology.fsu.edu/journal/hold.html&usg=__u3c6ftQ5E5GS0kI3MrKhh9ltTM=&h=531&w=497&sz=11&hl=en&start=20&zoom=1&tbnid=pwVUM_wWgJCOsM:&tbnh=132&tbnw=124&ei=KfyATYjYBZLugQe7hdGnBw&prev=/images%3Fq%3Dhold%2B1976%2Bsaliva%2Bformation%26um%3D1%26hl%3Den%26safe%3Dactive%26sa%3DN%26tbm%3Disch&um=1&itbs=1)

# Saliva - Oral Fluid Production

- Daily volume – up to 1,500 mL
- pH ~ 6.7 (blood 7.4)
- % water – 98% (plasma ~ 91%)

<b>Electrolytes</b>	<b>Saliva</b>	<b>Plasma</b>
K <sup>+</sup>	8-40	3.5-5.5
Na <sup>+</sup>	5-100	135-155
Cl <sup>-</sup>	5-70	100-110

# Saliva Formation

- Hold, 1996
- Figure 3
  - [http://www.google.com/imgres?imgurl=http://www.criminology.fsu.edu/journal/img00003.gif&imgrefurl=http://www.criminology.fsu.edu/journal/hold.html&usg=\\_\\_u3c6ftQ5E5GS0kl3MrKhu9ltTM=&h=531&w=497&sz=11&hl=en&start=20&zoom=1&tbnid=pwVUM\\_wWgJCOsM:&tbnh=132&tbnw=124&ei=KfyaTYjYBZLUgQe7hdGnBw&prev=/images%3Fq%3Dhold%2B1976%2Bsaliva%2Bformation%26um%3D1%26hl%3Den%26safe%3Dactive%26sa%3DN%26tbn%3Disch&um=1&itbs=1](http://www.google.com/imgres?imgurl=http://www.criminology.fsu.edu/journal/img00003.gif&imgrefurl=http://www.criminology.fsu.edu/journal/hold.html&usg=__u3c6ftQ5E5GS0kl3MrKhu9ltTM=&h=531&w=497&sz=11&hl=en&start=20&zoom=1&tbnid=pwVUM_wWgJCOsM:&tbnh=132&tbnw=124&ei=KfyaTYjYBZLUgQe7hdGnBw&prev=/images%3Fq%3Dhold%2B1976%2Bsaliva%2Bformation%26um%3D1%26hl%3Den%26safe%3Dactive%26sa%3DN%26tbn%3Disch&um=1&itbs=1)

# Constituents and Transfer Blood to OF

- Electrolytes ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and  $\text{HCO}_3^-$ ),  $\text{Li}^+$ , immunoglobulins, steroids, enzymes, DNA, viruses, bacteria, antibiotics...other drugs (ethanol, amphetamine, meth, SMA, barbiturates, benzodiazepines, cocaine, LSD, THC, opiates/opioids, PCP, nicotine)
- Active transport
- Diffusion – lipophilicity –  $\text{pKa}$
- Unbound – Not conjugated

# Drug Detection Times by Specimen

- Caplan, 2003
- Drug Detection in Alternate Specimens

Specimen	Time Frame
Blood	Minutes to hours
Saliva	Minutes to hours
Urine	Minutes to days
Sweat	Minutes to weeks
Hair and nails	Days to years

# OF Drug Testing Considerations: Sensitivity/Volume/Analyte

<b>Drug/Cutoff</b>	<b>Urine (ng/mL)</b>	<b>Oral Fluids (ng/mL)</b>
Amp/Meth	1000/500	50
Codeine/Mor	2000/300	40
Cocaine/BZE	300/150	2-8
THC	n/a	2
THCA	50/15	n/a
Benzodiazepines	50-100	1-2
Opioids	50-100	20-40
<b>Testing Volume</b>	>30 mL	<2 mL
<b>Analyte</b>	Metabolite/drug	Drug

# Oral Fluid - Clinical Study

- Determine if there is a predictable relationship between codeine concentrations in saliva/OF vs. plasma (NIST, 2004)
- 17 Subjects (M/F)
- Overnight stay in CRC
- Single 30 mg dose of codeine phosphate
- Brush teeth and rinse mouth
- Heparin blood and OF collected (spitting) @ 0, 15, 30 min and 1, 2, 4, 6, 8, 10, 12 and 24 hours
- Plasma harvested
- O'Neal, 1999

# Sample Analysis

- 0.5 mL of OF
- Calibration curve 5 to 500 ng/mL
- Codeine D3 and Morphine D3 (25 ng)
- Extract using solid phase extraction column
- Elute from column – evaporate solvent
- Derivatize with TFAA
- Analyze by positive ion chemical ionization
- GC/MS
- O'Neal, 1999

# Mean Concentrations OF and Plasma Codeine

Time, hours	Saliva Codeine, ng/mL	Plasma Codeine, ng/mL
0.25	4129	12
0.5	1172	38
1	480	37
2	154	34
4	60	18
6	36	9
8	19	5
10	11	4
12	7	2

# Mean OF/P Ratio

Time, h	Plasma, ng/mL	OF, ng/mL	OF/P Ratio
0	0	0	0
0.25	12	4129	344
0.5	38	1172	31
1	37	480	13
2	34	154	4.5
4	18	60	3.3

# Mean OF/P Codeine Ratio Over Time

Time, hours	Saliva/Plasma Ratio
0.25	344
0.5	31
1	13
2	4.5
4	3.3
6	4.5
8	3.8
10	2.8
12	3.5

# pH vs. OF/P Codeine Ratio

- O'Neal, 1999 & Crouch, 2005

pH	Actual OF/P ratios	Theoretical OF/P ratios	Mean OF/P ratios
6.0	2.8, 3.1, 3.5, 3.8, 4.2, 4.5, 5.7, 5.8, 6.8, 8.1, 10.8	20.0	4.7
6.5	1.7, 1.9, 2.8, 3.1, 3.7, 3.9, 4.7, 6.3, 6.6, 9.8		
7.0	1.1, 1.4 ,1.6, 1.9, 2.2, 2.5, 2.7, 3.0, 3.2, 3.4, 3.6, 3.9, 4.1, 4.4, 4.8, 5.4, 5.8, 6.2, 6.7, 7.6	2.0	3.4
7.5	3.1, 3.4, 4.4		
8.0	0.9, 1.2, 2.4, 4.1		1.8

# Study Conclusions

- OF could be collected simply, repeatedly, non-invasively & under observation
- A limitation of OF as a specimen for interpretation was oral contamination
- There was a reasonably predictable OF/P for codeine after 2 hrs
- Codeine concentrations in OF exceeded those in plasma
- pH did not have predicted effect

# Oral Fluid Collectors

# Clinical Study II

- Effect of collection technique (NIST, 2004)
  - Spitting
  - Stimulated – non acidic
  - Stimulated - acidic
  - Device - Salivette or Finger Collector
- n = 5 Subjects/collection technique
- Single 30 mg dose of codeine phosphate
- O'Neal, 2000

# Mean Codeine Time Course by Device

- O'Neal, 2000, Codeine concentrations, ng/mL

Time, h	Control	Acidic	Nonacidic	Salivette	Finger collector
0.25	3542	556	1365	1171	1013
0.5	1000	203	338	916	825
1	413	103	183	356	412
2	152	74	94	99	106
4	66	18	47	60	62
6	38	12	21	23	19
8	19	5	9	21	34
10	12	<5	6	7	7
12	8	ND	<5	<5	5

# Collection Method Comparison

- Crouch, 2004, ratio: Collector/Control

Time, h	Acidic	Nonacidic	Salivette	Finger collector
0.25	0.16	0.39	0.33	0.29
0.5	0.20	0.34	0.92	0.83
1	0.25	0.44	0.86	1.00
2	0.49	0.62	0.65	0.70
4	0.27	0.71	0.91	0.94
6	0.32	0.55	0.60	0.50
8	0.26	0.47	1.11	1.79
10		0.50	0.58	0.58
12				0.63

# Duration of Codeine Detection/Quantitation

Condition	$\geq 1$ ng @ 12 h (LOD)	$\geq 5$ ng @ 12 h (LOQ)	$\geq 1$ ng @ 24 h (LOD)
Control	100%	86%	68%
Salivette	100%	40%	40%
Finger C	100%	40%	20%
Non-acidic	60%	40%	40%
Acidic	20%	20%	0%

# Conclusions

- Stimulation reduces OF drug concentration(s) & affects drug detection and quantitation
- Spitting was the most effective collection technique. 3.6 X acidic, 2.0 X non-acidic, 1.3 X Salivette or Finger Collector
- Published OF-drug study results should be scrutinized. **How were the specimens collected?**

# Drug Recovery From Collectors

## (Average % vs. Control Pools)

- Crouch, 2004 & 2007 (NIST)

Drug	Hooded collector	Finger collector	Oralscreen	Salivette	Intercept
Amphet	28	55	28	56	34
Methamp	26	57	34	57	25
Codeine	47	41	35	45	35
Morphine	42	49	37	41	34
Cocaine	67	62	69	92	96
BZE	93	93	96	97	90
PCP	15	27	17	55	32
THC	<10	<10	<10	<10	<10
THCA	2	<1	2	31	51

# Quantisal™ Recovery vs. Other Collectors (Average %)

- Quintela, 2007

Drug	Low	Med	High	Range
Amphetamine	94	97	97	16-57
Methamphetamine	98	104	93	25-59
Codeine	100	96	104	33-49
Morphine	99	93	92	33-50
Cocaine	96	91	93	61-96
BZE	91	87	83	85-98
Oxazepam	109	101	98	n/a
Methadone	101	100	107	n/a
THC	84	91	81	<10

# OF Specimen Validity - IgG?

- Crouch 2004 & 2005
- \* Initial mean = 2.67

Statistic	IgG	Rinse 1	Rinse 2
N	100	6	6
Mean, mcg/mL	2.98	2.33*	1.99
% Decrease	n/a	12.7%	25.5%
S.D.	0.58	0.77	0.57
S.D. , mean to fail	~4.2	~2.3	~2.6

# Discussion

- Define the specimen & Volume – saliva/OF, etc
- Collection method(s) – device & criteria; spitting
- Cutoffs, drugs/metabolites and methods of analysis
- Specimen validity - volume/weight; chemical marker(s); stimulation
- Focus on advantages:
  - Observed collection & no special facilities requirements
  - Potential relationship to blood concentrations
  - Easily transported
  - Easily analyzed
  - Complementary to urine, hair, sweat patch, blood, etc.

**Thank you for your attention!!**