Alternative Specimens for Drugs of Abuse Testing in Pain Management: Oral Fluid

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Learning Objectives

After this presentation, you should be able to:

1. Describe how drugs enter oral fluid and the basic dynamics of saliva/plasma concentration ratios
2. List some options for oral fluid sample collection and potential impact of collection devices on the sample.
3. Describe testing methodologies that can be used for identification of pain management drugs in oral fluid.
4. Summarize some advantages and disadvantages inherent in the use of oral fluid as an analytical sample.

Introduction

While urine is the most commonly used test matrix for compliance monitoring in pain management programs, interest in alternate matrices, including oral fluid is growing. The ability to perform observed, non-invasive sample collection is a primary driver. In addition, oral fluid levels and detection periods for many drugs more closely align with plasma which may provide better insight into patient compliance with dosing protocols.

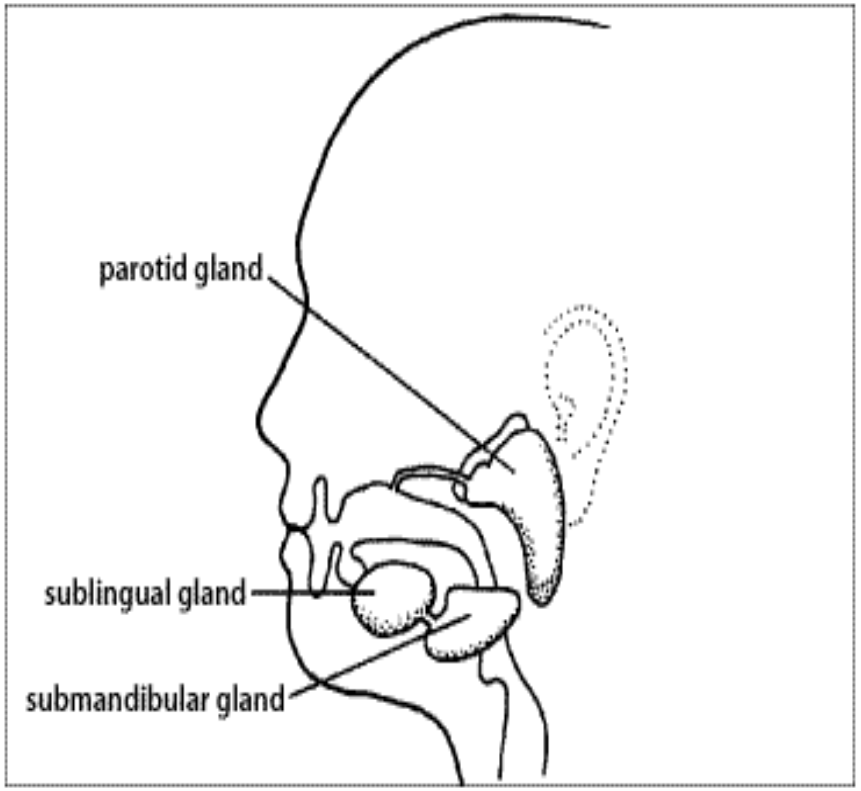
This presentation will include an overview of oral fluid as an analytical sample, advantages and disadvantages of oral fluid testing for pain management, and current approaches for implementing oral fluid testing in clinical laboratories.

Oral fluid as an analytical sample

Oral Fluid as an Analytical Sample

Saliva Physiology

- Colorless fluid, pH 6.7, secreted into oral cavity by salivary glands (parotid, submandibular, sublingual).
- Human saliva is 99% water. Electrolytes, mucus, glycoproteins, enzymes, immunoglobulins and biomarkers are also present.
- Under normal healthy conditions, adults produce 0.5 - 1.5 L of saliva per day.
- Flow rates vary throughout the day and with stimulation (e.g. chewing). Secretion can also be affected by medications.
  - *Reference: Aps, JKM and LC Martens, Forensic Science International 150 (2005), pp 119 – 131.*



Oral Fluid as an Analytical Sample

Terminology: Oral Fluid vs. Saliva

- Oral Fluid is sometimes called 'mixed saliva' and represents all of the fluid in the mouth
  - Secretions from major and minor salivary glands + gingival crevices + mucous + cellular debris[1,2]
- The terms are often used interchangeably, however, *oral fluid* better represents the analytical specimen

- *1. Malamud, D. Guidelines for saliva nomenclature and collection, Ann NY Acad Sci 694 (1993), pp. xi – xii*
- *2. Schramm, W. RH Smith and PA Craig, Ann NY Acad Sci 694, September 1993, pp 311 – 313.*

Oral Fluid as an Analytical Sample

Drug Deposition

- Drugs appear in saliva soon after dosing, predominately by passive diffusion from the blood. Oral fluid drug concentrations generally reflect the free (unbound) fraction of the drug in plasma
- Physiochemical properties of the drug and saliva pH determine final drug concentrations
  - Variables include pH, p*K*a, lipid solubility, charge, molecular weight, protein binding, salivary flow rate
- Parent drugs often predominate in OF because they tend to be more lipid soluble which facilitates movement through capillary cell membranes

Reference: Aps, JKM and LC Martens, Forensic Science International 150 (2005), pp 119 – 131.

Oral Fluid as an Analytical Sample

Saliva/Plasma (S/P) Ratios

- Partitioning of drug between oral fluid and plasma is often expressed as the Saliva/Plasma Ratio (S/P); an S/P ratio of 1:1 indicates that drug concentration in both compartments is equal
- Because oral fluid tends to be slightly more acidic than blood, basic drugs ionize and concentrate in oral fluid (higher S/P ratios) due to "ion trapping". Weakly acidic and more highly bound drugs are present in lower concentrations.[1]
- Opioid compounds prescribed for pain management including oxycodone, hydrocodone and tramadol have high S/P ratios and are easily measured in oral fluid.[2]

[1] *Aps, JKM and LC Martens, Forensic Science International 150 (2005), pp 119 – 131.*
[2] *Moore, Christine, Journal of opioid management 11(1): 69 -75, January/February 2015*

Oral Fluid as an Analytical Sample

Average Oral Fluid/Blood Ratios

Drummer, O., Clin Biochem Rev 27(3): 147-159, 2006

Drug	Average S/P ratio
Ethyl Alcohol	1.07
Barbiturates	0.3
Buprenorphine	1
Codeine	4
Methamphetamine	2
MDMA	7
Cocaine	3
Diazepam	0.02
Methadone	1.6
Morphine	0.8
THC	1.2
Tramadol	9

Note: S/P ratios will vary with route of administration, saliva pH, and other factors

Oral Fluid as an Analytical Sample

Detection Times of Drugs in Oral Fluid

Sources: Verstraete, A., Ther Drug Monit 26(2): 200 – 205, 2004; Bosker, WM and Huestis, M, Clin Chem 55(11): 1910 – 1931, 2009

Drug	Detection Time (hrs)
Amphetamine/Methamphetamine	20 – 48
MDMA	24
Barbiturates	50
Benzodiazepines	5 - 50
Cocaine/BZE	12/48
THC	2 - 24
Codeine/Morphine/Hydrocodone/Oxycodone	12 - 24
6-Acetylmorphine	0.5 - 8
Methadone	24
Buprenorphine	13 - 28

Oral Fluid as an Analytical Sample

Current Applications

- While urine has been the specimen of choice for many drug screening programs, interest and use of oral fluid as an analytical sample is growing.
- Workplace Drug Testing
  - Non-regulated; Guidelines for regulated testing pending
- Driving Under the Influence of Drugs
  - Rosita, DRUID studies (on-site devices)
- Compliance
  - Smoking cessation (cotinine)
  - Substance Abuse Treatment
  - Pain management

Oral Fluid as an Analytical Sample

Prevalence and Positive Rates: Workplace

Quest Diagnostics Drug Testing Index™ , oral fluid/urine comparison data

Reported Overall Positive Rates, Quest DTI, General Workforce

Year	Oral Fluid % Positive	Urine % Positive
2010	4.4	4.2
2011	4.3	4.1
2012	5.5	4.1
2013	6.7	4.3
2014	7.7	4.7

Source: Quest Diagnostics.com/DTI, Spring 2015 report

Journal of Analytical Toxicology 2002[1]

Positive Prevalence Rates for 77,218 oral fluid specimens tested at a SAMHSA-certified laboratory

- Overall positive rate of 5.06%, similar to contemporaneous urine data

[1] *Cone, EJ, et.al. J Anal Toxicol 2002 Nov-Dec; 26(8): 541 – 546*

Oral Fluid as an Analytical Sample

MRO Positive Rate Data Comparison

The Walsh Group, DTAB 01/2011 http://162.99.3.215/DTAB/presentations.aspx

- Data from approximately 2 million urine and 650,000 oral fluid tests compared
- All test results from single MRO source, oral fluid samples from primarily 2 large laboratory systems, non-regulated workplace

Matrix	Urine	Oral Fluid
% Lab Confirmed Positives	4.15	4.30
% MRO Verified Positives	76.4	95.6
% MRO Reversals	23.6	4.4

Oral Fluid as an Analytical Sample

Prevalence and Positive Rates: Pain Management

Journal of Analytical Toxicology 2011

Oral Fluid Drug Testing of Chronic Pain Patients: Positive Prevalence Rates of Licit and Illicit Drugs

6441 oral fluid specimens analyzed for 14 drug classes

- Overall OF non-negative screening rate was 83.9% compared to 78% for urine
- Prevalence of confirmed positive drug groups was similar to urine
- 11.5% of study population used 1 or more illicit drugs, compared to 10.9% of urine donors

Heltsley, R, et.al. J Anal Toxicol 2011; 35(8): 529 - 540

Journal of Analytical Toxicology 2012

Oral Fluid Drug Testing of Chronic Pain patients: Comparison of paired oral fluid and urine specimens

133 chronic pain patients, 1544 paired tests

- Overall qualitative agreement 85%
- Discordant results: 5.4% positive in OF, negative in UR, 9.6% positive in UR, negative in OF
- Overall agreement increased to 89% when only DHHS drug categories (including hydrocodone and oxycodone) were included

Heltsley, R, et.al. J Anal Toxicol 2012; 36(2): 75 - 80

Oral Fluid as an Analytical Sample

Detection of Heroin Use

- Oral fluid may provide improved identification of heroin use; studies show higher prevalence of 6-AM in oral fluid than in urine

  Opiate oral fluid concentrations in specimens (n = 62) collected from heroin-dependent pregnant women from: Dams, R. et.al. Drug Alcohol Depend 87(2-3): 258 – 267, 2007.

Analyte	Prevalence
Heroin	41.9%
6-AM	72.6%
Morphine	25.8%
Codeine	12.9%
Acetylcodeine	9.7%

Oral Fluid as an Analytical Sample

Prevalence and Positive Rates

- While data shows comparable positive rates in urine and oral fluid, results should be interpreted carefully
- Studies use different collection, testing methods and cutoffs; equivalence between urine and oral fluid cutoffs not clearly established
- Data supports the viability of oral fluid as an alternate matrix in drug testing programs

Oral Fluid Testing Procedures

Oral Fluid Testing Procedures

Specimen Collection

- Oral fluid may be collected by expectoration, but the most common method is by use of a collection device with a pad and a transport tube with buffer solution
- Buffers in the devices stabilize samples, facilitate drug recovery from the collection pad and prevent bacterial growth during transportation and storage.
- A variety of devices are available; reagent-specific

Oral Fluid Testing Procedures

The Collected Sample

- Collection methodology and devices are a significant source of variability in oral fluid testing [1]
  - Stimulated vs unstimulated, drug recovery, specimen volumes (qualitative vs quantitative)
  - FDA requires clearance of the test with a collector
- If using a collection device that contains a buffer, the actual analytical sample is oral fluid diluted 2 – 4x
- Assays designed to compensate for dilution factor

[1] *Crouch, Dennis J. Forensic Science International 150: 2-3, 165 – 173, June 2005.*

Oral Fluid Testing Procedures

Specimen Collection

- Limited sample volume collected
  - particularly important consideration for applications with multiple positive results
- Most quantitative devices collect 1.0 mL of oral fluid
  - Testing methodologies must be optimized for small sample volumes and lower concentrations
  - MS instrumentation and assays optimized for small sample size have become more common in clinical laboratories
- Drug recovery from the collection pad may vary by device

Oral Fluid Testing Procedures

Collection Devices with Cleared Tests – Laboratory-based testing (04/2016)

Vendor	Format	Collection Device	Buffer/Neat	Dilution
Orasure	ELISA	Intercept	Buffer	approx 3
Roche	HEIA	Intercept	Buffer	approx 3
Immunalysis	ELISA	Quantisal	Buffer	4
Lin-Zhi	HEIA	Lin-Zhi	Neat	NA
Lin-Zhi	HEIA	Salivette	Neat	NA
Microgenics	HEIA	Oral-Eze	Buffer	3
Biophor	Fluorescence	RapidEase	Neat	NA

Advantages and Disadvantages

Specimen Collection

Advantages:

- Primary driver for use of oral fluid in drug testing programs
  - Ease of Collection
  - Non-invasive, minimal infrastructure required
  - Collections are observed, minimizing opportunity for specimen adulteration
  - Gender-neutral
  - Specimens can be collected in 5 -7 minutes
  - Buffer in devices provides stability, assists in recovery

Disadvantages/Considerations:

- Depending on device, collection volumes can be inconsistent
  - Newer devices have volume indicators, improved reproducibility
- Drug Recovery from collection pads
  - Improving; Reported THC recoveries > 80%; may vary by device
- Medications affecting salivary secretion can lead to extended collection times
- Stimulated collections can change pH and drug concentrations in OF
- Added cost

Oral Fluid Testing Procedures

Testing Methodologies

- Considerations: Limited specimen volume and lower drug concentrations as compared to urine
- Screening Methodologies:
  - Immunoassays commercially available- ELISA or Homogeneous EIA
    - Assays may be modified for improved cross-reactivity with parent drug
    - Limited menu of FDA-cleared assays; wider availability of RUO/FUO tests
  - Multicomponent LCMSMS methodology
- Confirmation testing by chromatography w/ mass spectrometry (HPLC/MS/MS prevalent)

Oral Fluid Testing Procedures

FDA-Cleared Oral Fluid Tests as of 12/2015 (lab-based, US Market)

Source: http://www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/510kClearances/default.htm

Vendor	Tests	Format	Collection Device	Cleared
Orasure	AMP, MAMP, THC, COC, OPI, PCP, BARB, BENZ, MDONE	ELISA	Intercept	2000 - 2002
Immunalysis	AMP, MAMP, COC, OPI	ELISA	Quantisal	2005
Roche	AMP, MAMP, COC, OPI, PCP	HEIA	Intercept	2011
Lin-Zhi	AMP, MAMP, THC, 6AM	HEIA	Lin-Zhi	2014-2015
Lin-Zhi	AMP, MAMP, OPI, COC,MDONE	HEIA	Salivette	2006
Microgenics	AMP, MAMP, THC, COC, OPI, PCP	HEIA	Oral-Eze	2011
Biophor	AMP, MAMP, MDMA, THC, COC, OPI, PCP	Fluorescence	RapidEase	2013 - 2015

Oral Fluid Testing Procedures

Detection Limits and Cutoffs

- Immunoassay screening cutoffs vary by test type and manufacturer
  Examples of FDA-Cleared Product Cutoffs
  http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm

Test	Orasure	Immunalysis	Roche	Lin-Zhi	Microgenics	Biophor	2004 SAMHSA	2015 SAMHSA
AMP	300	50	120	50	150	50	50	25
MAMP	120	50	120	50	120	50	50	25
MDMA						50	50	25
THC	3			4	3	4	4	4
COC	15	20	9		15	20	20	15
OPI	30	40	30		30	40	40	30
6-AM				4			4	3
PCP	3		6		3	10	10	3

Oral Fluid Testing Procedures

Detection Limits and Cutoffs

- Definitive testing detection limits (MS, MSMS LDT's) are optimized for target drugs; range from pg/mL – high ng/mL
- Can be used for screening and/or confirmatory testing
- Numerous publications of validated methods for the detection of drugs relevant to pain management in oral fluid.
- Predominant technique is LCMSMS; simultaneous analysis of compounds of different polarity, without derivatization, good sensitivity

Oral Fluid Testing Procedures

Drug and Metabolite Concentrations in Oral Fluid from Pain Patients – LCMSMS

Heltsley, R, et.al. J Anal Toxicol 2011; 35(8): 529 - 540

Drug	N	Median (ng/mL)
Amphetamine	190	179.8
Diazepam	396	5.5
Nordiazepam	307	11.9
Alprazolam	582	11.1
Clonazepam	35	5.2
Buprenorphine	122	16.0
Meprobamate	439	1543.5
THC	396	11.0
Methadone	462	63.8

Drug	N	Median (ng/mL)
Fentanyl	424	6.6
Codeine	136	7.5
Morphine	619	18.1
Hydrocodone	1843	67.8
Hydromorphone	304	2.7
Dihydrocodeine	1600	7.8
Oxycodone	1847	120.1
Noroxycodone	1952	48.5
Tramadol	149	237.6

Advantages and Disadvantages

Testing Methodologies

- Immunoassays are available; menu of FDA-cleared tests is limited.
- Tests must be optimized for relevant analytes, lower concentrations.
- Lack of standardization among vendors with regard to cutoffs.
- MS instrumentation requirements may be a barrier for some laboratories.

- Wider availability of immunoassays available as *'forensic use only'.* More tests in development.
- Technologies are evolving; more immunoassays available in homogeneous format.
- Validated methods using LCMSMS or other chromatographic technologies widely published.
- Introduction of oral fluid testing to facilities already performing toxicology testing not difficult.

Oral Fluid Testing Procedures

Interpretation of Results

- Several studies have attempted to correlate oral fluid with plasma concentrations for therapeutic or dosing studies with mixed results.
- In general, oral fluid results are temporally correlated with plasma however concentrations are dependent on lipophilicity and pKa of the drug.[1]
- Buccal cavity contamination from smoked, inhaled, or sublingually-administered drugs can skew data; concentrations equilibrate over time.[2]
- For compliance purposes, numerous studies support the relative equivalence of oral fluid vs urine as the analytical sample.
- Parent drugs predominate, which may provide a better indicator of recent use

[1] *Dams, R. et.al. Drug Alcohol Depend 87(2- 3): 258 – 267, 2007.*
[2]*Moore, Christine, Journal of opioid management 11(1): 69 -75, January/February 2015*

Oral Fluid in Pain Management

Summary – Compliance Monitoring

- Drugs perfuse from plasma into oral fluid; levels depend on chemical properties of the drug
- Basic drugs, including many pain management drugs, have higher concentrations in oral fluid than plasma due to 'ion trapping'. More acidic and highly protein-bound drugs are present in lower concentrations.
- Detection times of drugs in oral fluid are similar to plasma, however prevalence studies show positive rates similar to urine
- The presence of parent drug in oral fluid may assist in evaluation of treatment compliance

Oral Fluid in Pain Management

Summary – laboratory testing

- Specimen collection devices and procedures are an important component of the testing process and can impact drug detection.
- Commercial immunoassays are available for oral fluid testing, however, the current menu of FDA-cleared tests is limited and testing thresholds are inconsistent.
  - Inclusion of oral fluid in HHS Workplace Drug Testing Programs will expand the market and force standardization
  - A wider assay menu is available as FUO
- Limited specimen volume and lower drug concentrations require optimization of testing methods. Numerous publications of validated chromatographic/ MS methods; LCMSMS predominates.

To Use or Not to Use?

- Oral fluid is a valid matrix for compliance testing in pain management programs.
- As with any matrix, there are advantages and disadvantages, strengths and limitations that should inform the decision.
- Implementation of testing in a laboratory already providing toxicology services can be relatively straightforward.

Thank you for attending!

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Self-Assessment Questions

1. Which of the following is not a true statement?
   - a. Drugs enter oral fluid via passive diffusion.
   - b. Drugs in oral fluid are always in equal concentrations to plasma.
   - c. Parent drugs are detected more often in oral fluid than in urine.
   - d. Basic drugs, including those important in pain management, can concentrate in oral fluid due to ‘ion trapping’.

Self-Assessment Questions

2. Oral fluid collection devices can have an impact on the sample because:
   a. Buffers in devices may cause drugs in the sample to deteriorate.
   b. Some drugs may be difficult to recover from collection pads.
   c. Insufficient volume may be collected in devices without volume indicators.
   d. a, b, and c are correct
   e. b and c are correct

Self-Assessment Questions

3. Which of the following are considered advantages in use of oral fluid as an analytical sample?

- a. Oral fluid collections are observed and thus less susceptible to sample adulteration or substitution
- b. Detection *periods* are similar to plasma, however prevalence data indicates that detection *rates* are similar to urine
- c. Oral fluid has lower drug concentrations than urine which makes it easier to adapt testing technologies for use in this matrix.
- d. Cutoffs for FDA-cleared reagent kits are standardized regardless of vendor
- e. All are correct
- f. Only a and b are correct