Oral fluid
An upcoming matrix for drugs of abuse testing

Michaela Neuhofer
Introduction

- Kind of glands
- Function of the glands
- Production of saliva
- Properties of saliva and oral fluid
- Oral fluid collection
- Transportation of drugs into oral fluid
- Matrix authenticity
in saliva veritas - History

- 1690 Antonius Nuck
- 1856 Claude Bernard
- 1932 Amberson and Höber
- > 1970’s Young and Van Lennep
  - Aps
  - Antoon Ligtenberg
  - Aps Crouch
  - Vissink
  - Sam Niedbala
  - Marilyn Huestis
  - Michael Böttcher
  - Brumagen
  - David Wong
  - Schramm
  - Guy Carpenter
  - Robert Flanagan
  - Olof Beck

<table>
<thead>
<tr>
<th></th>
<th>Google</th>
<th>PubMed</th>
</tr>
</thead>
<tbody>
<tr>
<td>saliva</td>
<td>19,100,000</td>
<td>42,719</td>
</tr>
<tr>
<td>oral fluid</td>
<td>8,130,000</td>
<td>22,914</td>
</tr>
<tr>
<td>oral fluid drug testing</td>
<td>6,680,000</td>
<td>565</td>
</tr>
</tbody>
</table>
Protection of teeth and mucous membrane
- Electrolytes and mucine

Immunological function
- IgA

Antibacterial protection
- Lysocym, Lactoferin,…

Part of the digestive system
- Alpha-Amylase
The anatomical location of salivary glands:
1 Glandula parotis, ~ 20 - 23%
2 Glandula submandibularis, ~ 65 - 70 %
3 Glandula sublingualis, ~ 4 - 5 %
4 Glandula minor glands ~5 -8 %

Types of saliva
*Parotid saliva (1):* serous-secreting cells
*Submandibular saliva (2):* predominantly serous and some mucin secreting cells
*Sublingual saliva (3):* more mucous, less serous acini
*Minor glands:* mixed serous and mucous acini
*Oral Fluid:* low viscosity and low elasticity (slightly ropy, fairly low viscosity)
Acinar cells: first stage primary salivary fluid
- Cl⁻ influx by electrochemical gradient

Duct cells: second stage
- ionic composition is modified by NaCl reabsorption and secretion of K⁺ and HCO₃⁻,
- Osmotic pressure of OF is a sixth of that of plasma

Buffer and pH
- Bicarbonate buffer: \( \text{HCO}_3^- + \text{H}^+ \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}_2\text{O} + \text{CO}_2 \)
  - pH optimum 6.1
- Phosphate buffer: \( \text{HPO}_4^{2-}/\text{H}_2\text{PO}_4 \)
  - pH optimum 7.1
- Protein buffer
General considerations
- time of the day
- season
- duration of collection
- natural condition (hormone status,..)
- gland specific variations

Non stimulated collection of whole saliva means draining method
- avoid any kind of stimuli
- be seated comfortably
- eyes open
- head slightly forward
- rest for 5 min
- minimize oral movement

(Wong: Saliva collectors 2008, C 4, Wolff Vissink, Veerman)
Stimulated saliva collection

- spitting method
- absorbents (swabs/pads)
- suction
- paraffin wax
- chewing gum
- gustatory stimuli
- liquids
- or ...
Mean contribution, expressed as a % of the total of the different salivary glands to the total salivary production according to a certain type of stimulation

<table>
<thead>
<tr>
<th>Glands</th>
<th>Sleep</th>
<th>No stimulation (resting OF)</th>
<th>Mechanic. stimulation (swab, pad, spitting)</th>
<th>Citric acid stimulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gl. parotis</td>
<td>0</td>
<td>21</td>
<td>58</td>
<td>45</td>
</tr>
<tr>
<td>Gl. submandibularis</td>
<td>72</td>
<td>70</td>
<td>33</td>
<td>45</td>
</tr>
<tr>
<td>Gl. sublingualis</td>
<td>14</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Minor saliv. glands</td>
<td>14</td>
<td>7</td>
<td>7</td>
<td>8</td>
</tr>
</tbody>
</table>

(Johan K.M. Aps, Luc C. Martens: The physiology of saliva and transfer of drugs into saliva, 2005)

Remember: gingival fluid is similar to that of plasma and thus it provides a potential route for the entry of many drugs into saliva.
Oral Fluid
pH between 5.8 and 7.6

DNA, RNA, proteins, pathogens, lipids and low-molecular components
= ultrafiltrate of blood

The transfer of analytes from blood to oral fluid can happen:

- Passive diffusion through cell membranes (liposoluble substances e.g. drugs or steroids)
- Active transportation (proteins like sIgA)
- Ultrafiltration (small polar molecules e.g. creatinine)
Transfer is influenced by different factors:

- Chemical properties of the drug
- protein-binding of the drug
- Ionization
- pKa of each drug
- basic or weak acidic compounds
- Lipophilicity

pH of oral fluid during collection

(Feller and le Petit, 1977; Ritschel and Thompson, 1983; Levy and Lampman, 1975, Cone and Huesis, 2007,...)
in saliva veritas – transport of drugs


Fig. 1. Schematic diagram for transport of drugs into saliva or sweat.
## in saliva veritas – transport of drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Protein-binding</th>
<th>$pK_a$</th>
<th>saliva/plasma ratio (S/P)</th>
<th>Concentration free drug OF vs. plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphetamine</td>
<td>15 – 30 %</td>
<td>10</td>
<td>2.8</td>
<td>OF &gt; P</td>
</tr>
<tr>
<td>Methamphetamine</td>
<td>10-20 %</td>
<td>10.1</td>
<td>2 – 7*</td>
<td>OF &gt; P</td>
</tr>
<tr>
<td>Cocain (Benzoylecognin, Ecoginmethylester)</td>
<td>80 – 85 %</td>
<td>8.8</td>
<td>0.4 – 9.7*</td>
<td>OF &gt; P</td>
</tr>
<tr>
<td>Heroin (Morphin, 6-AM)</td>
<td>70 %</td>
<td>7.6</td>
<td>0 – 789*</td>
<td>OF &gt; P</td>
</tr>
<tr>
<td>Benzodiazepine</td>
<td>70 - 97 %</td>
<td>3.3</td>
<td>1.8 - 13</td>
<td></td>
</tr>
<tr>
<td>THC</td>
<td>99 %</td>
<td>10.6</td>
<td>1.2</td>
<td>---</td>
</tr>
</tbody>
</table>

* IV or oral
(Verstraete 2004 und 2012; Cone and Huestis, 2007)
Proof of authenticity of the sample
= a combination of different factors
  – salivary amylase
  – cortisol
  – amount of collected sample
  – % of oral fluid
α-Amylase: > 10 000 U/L
besides digestion also an indicator for sympathetic nervous system activity

“OF Amylase concentration in PG (methadone substituted patients) showed no gender or age dependent differences. Even though YG (young group) database is small we assume no difference between PG and "normal population". Reference range OF Amylase (5% to 95%) taken from PG: 23000 - 433000 U/L 167 (41) samples from PG were suspected of substitution (4.9% (1.2%), Amylase <23000 U/L. From these spls. 23 (13.8%) revealed low %OF concentration.”
Cortisol, free ranges during day 4 - 15 ng/ml; evening/night 0,3 - 1,5 ng/ml

“Cortisol values in YG ranged from 1.03-5.93 ng/mL (5% to 95% percentile) and were much lower in PG with a skewed distribution (0.12 - 5.94 ng/mL . 5% to 95% percentile). Ten samples from PG had Cortisol levels below 0.12 ng/mL but eight had normal Amylase concentrations.”
Determination of % oral fluid

Male: 4.15 mL -> 47.9 %
Female: 4.02 mL -> 45 %

Young group: 6.79 mL and mean 59 %

Michael Böttcher  MVZ Labor Dessau GmbH
Determination of the amount of oral fluid in the collected sample

“The reference range for OF volume NG (380 random samples from healthy volunteers) and gave values from **3 mL to 5.5 mL** (5% to 95% percentile).”

%OF concentrations in the reference groups were normally distributed at about 59% (NG)

PG revealed a skewed distribution (mean 56%)

⇒ guarantee the individual specific quantification of collected OF

Michael Böttcher  MVZ Labor Dessau GmbH
Oral fluid enables

- **simple, painless, non-invasive** sample collection, gender-independent
- Possible to take **several repeat** saliva samples

In comparison to urine

- **adulteration** is prevented as collection can be directly observed
- authenticity provable by different markers
- give an indication of impairment

For analytics issues

- parent drugs, metabolites and especially important for new drugs (legal highs)
THANK YOU