Your Power for Health

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# Oral fluid

# An upcoming matrix for drugs of abuse testing


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# Introduction

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- 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**

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- 1690 Antonius Nuck
- 1856 Claude Bernard
- 1932 Amberson and Höber
- > 1970's Young and Van Lennep

Aps
Michael Böttcher
Olaf Drummer
Edward Cone
Robert Flanagan
Alain Verstraete
David Wong
Marilyn Huestis
Antoon Ligtenberg
Schramm
Sam Niedbala
Vissink
Guy Carpenter
Kim Grröschl
Nieuw Amerongen
Crouch
Andy Wolff
Olof Beck

| | Google | PubMed |
|---|---|---|
| saliva | 19.100.000 | 42719 |
| oral fluid | 8.130.000 | 22914 |
| oral fluid drug testing | 6.680.000 | 565 |

# in saliva veritas - **Function**

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- Protection of teeth and mucous membrane
  - Electrolytes and mucine
- Immunological function
  - IgA
- Antibacterial protection
  - Lysocym, Lactoferin,…
- Part of the digestive system
  - Alpha-Amylase

# in saliva veritas - **Glands**

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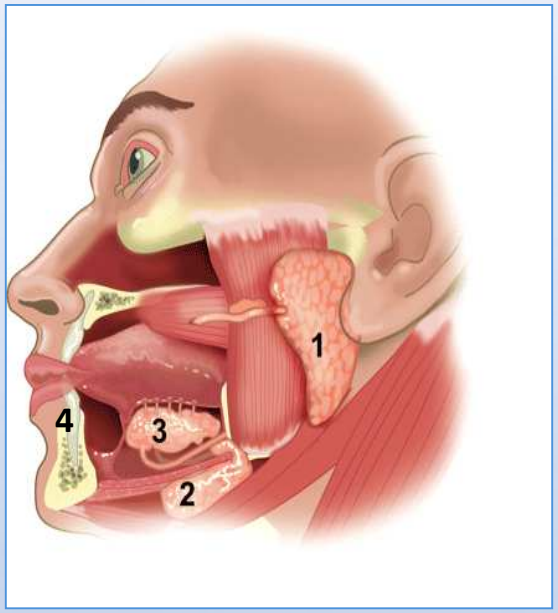
- The anatomical location of salivary glands:

  1 Glandula parotis, ~ 20 - 23%

  2 Glandula submandibularis, ~ 65 - 70 %

  3 Glandula sublingualis, ~ 4 - 5 %

  4 Glandula minor glands ~5 -8 %



(Van Nieuw Amerongen, 2004)

- 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)

# in saliva veritas – **salivary fluid**

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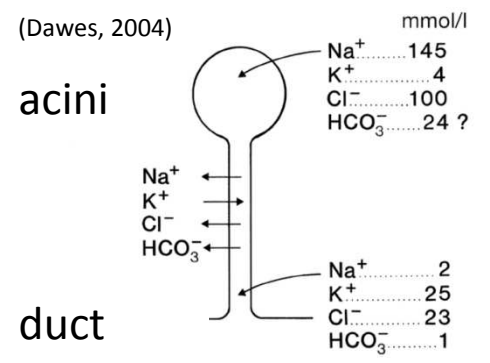


- Acinar cells: first stage primary salivary fluid
  - $Cl^-$ influx by electrochemical gradient
- Duct cells: second stage
  - ionic composition is modified by NaCl reabsorbtion and secrection of K+ and HCO3,
  - Osmotic pressure of OF is a sixth of that of plasma
- Buffer and pH
  - Bicarbonate buffer: $HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$
    - pH optimum 6.1
  - Phosphate buffer: $HPO_4^{2-}/H_2PO_4$
    - pH optimum 7.1
  - Protein buffer

# in saliva veritas - **Collection**

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- **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)

# in saliva veritas - **Collection**

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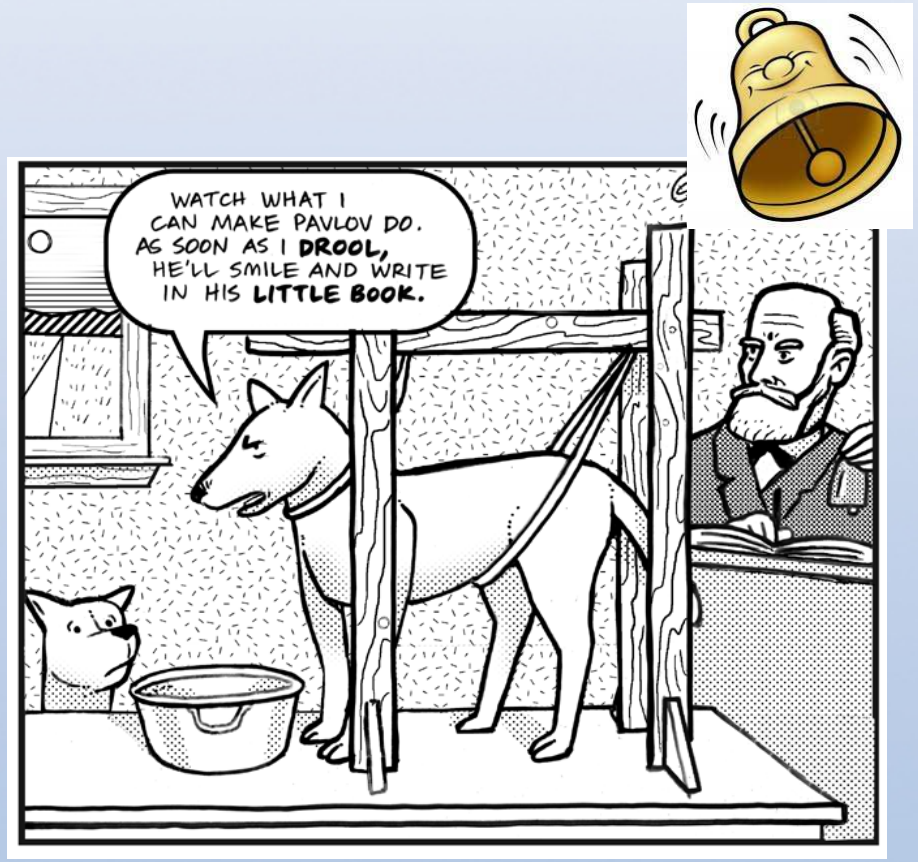
- Stimulated saliva collection
  - spitting method
  - absorbents (swabs/pads)
  - suction
  - paraffin wax
  - chewing gum
  - gustatory stimuli
  - liquids
  - or …



# in saliva veritas- **Collection**

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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

| Glands | Sleep | No stimulation (resting OF) | Mechanic. stimulation (swab, pad, spitting) | Citric acid stimulation |
|---|---|---|---|---|
| Gl. parotis | 0 | 21 | 58 | 45 |
| Gl. submandibularis | 72 | 70 | 33 | 45 |
| Gl. sublingualis | 14 | 2 | 2 | 2 |
| Minor saliv. glands | 14 | 7 | 7 | 8 |

(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

# in saliva veritas- **Oral fluid**

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**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)

# in saliva veritas –**transport of drugs**

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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
- Lipophicility

- 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**

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D.A. Kidwell et al. / J. Chromatogr. B 713 (1998) 111–135

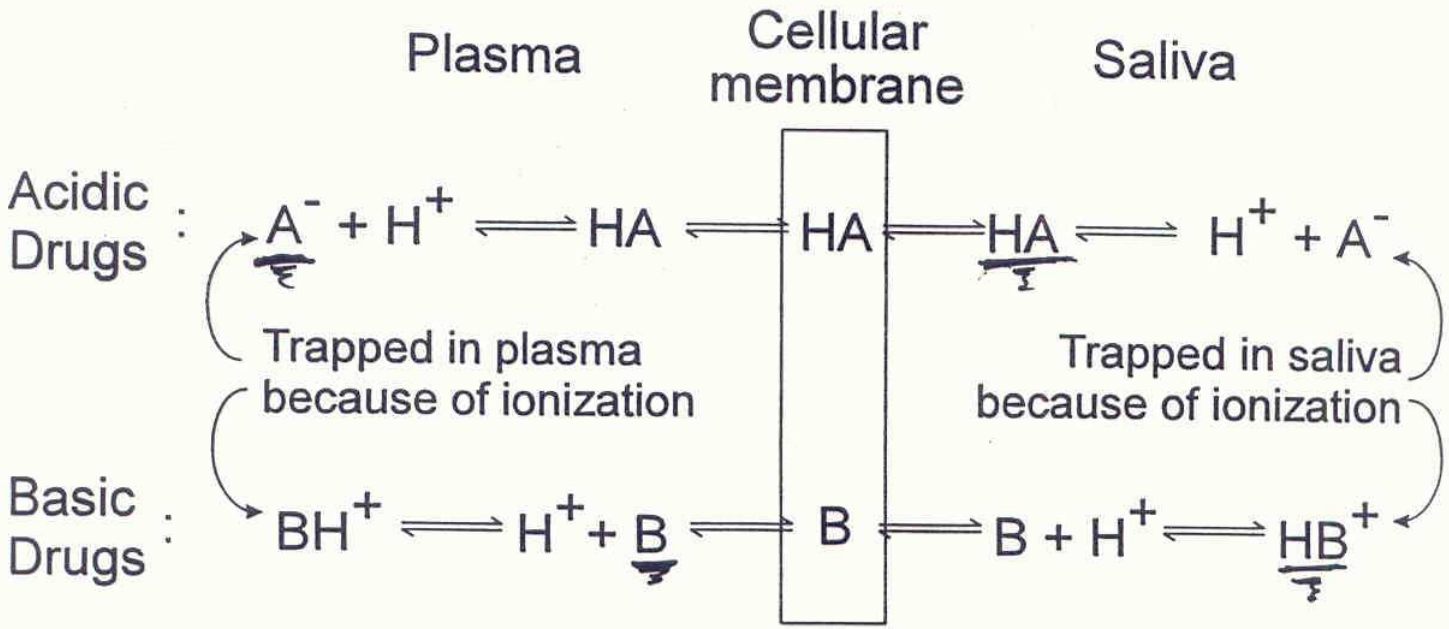


Fig. 1. Schematic diagram for transport of drugs into saliva or sweat.

# in saliva veritas –**transport of drugs**

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| | Protein-binding | $pK_a$ | saliva/plasma ratio (S/P) | Concentration free drug OF vs. plasma |
|---|---|---|---|---|
| **Amphetamine** | 15 – 30 % | 10 | 2.8 | OF > P |
| **Methamphet.** | 10-20 % | 10.1 | 2 – 7* | OF > P |
| **Cocain (Benzoylecognin, Ecoginmethylester)** | 80 – 85 % | 8.8 | 0.4 - 9.7* | OF > P pH of OF sample! |
| **Heroin (Morphin, 6-AM)** | 70 % | 7.6 | 0 – 789* | OF > P pH of OF sample! |
| **Benzodiazepine** | 70 - 97 % | 3.3 | 1.8 - 13 | |
| **THC** | 99 % | 10.6 | 1.2 | --- |

* IV or oral
(Verstraete 2004 und 2012; Cone and Huestis, 2007)

# in saliva veritas - **Sample authenticity**

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- Proof of authenticity of the sample
- = a combination of different factors
  - salivary amylase
  - cortisol
  - amount of collected sample
  - % of oral fluid

# in saliva veritas - **Sample authenticity**

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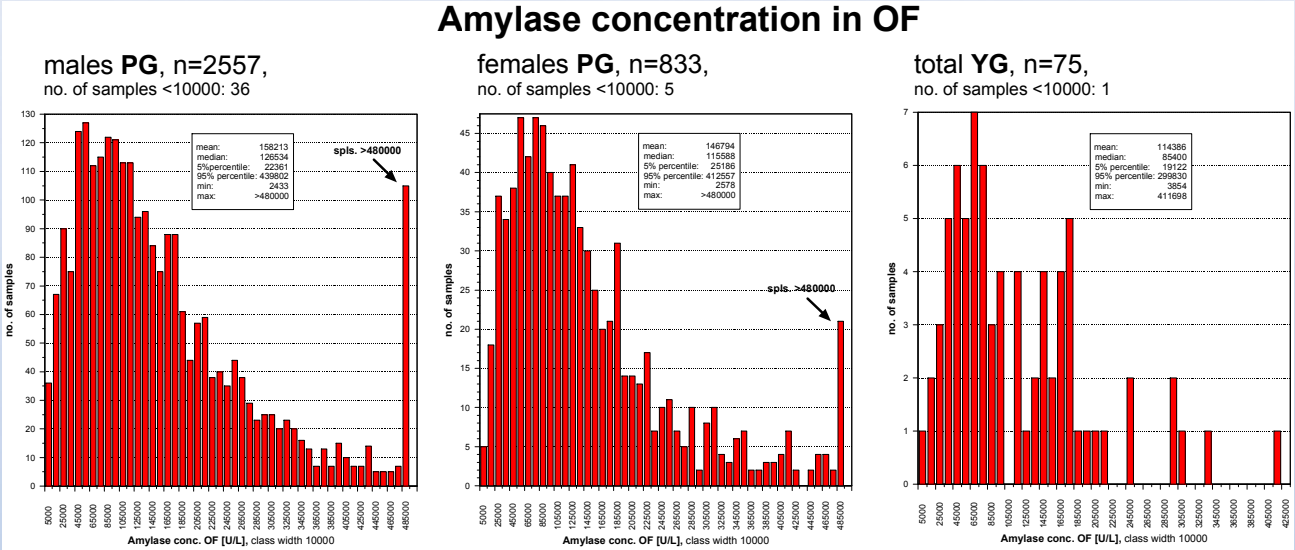
α-Amylase: > 10 000 U/L
besides digestion also an indicator for sympathetic nervous system activity




"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."

Michael Böttcher MVZ Labor Dessau GmbH

# in saliva veritas - **Sample authenticity**

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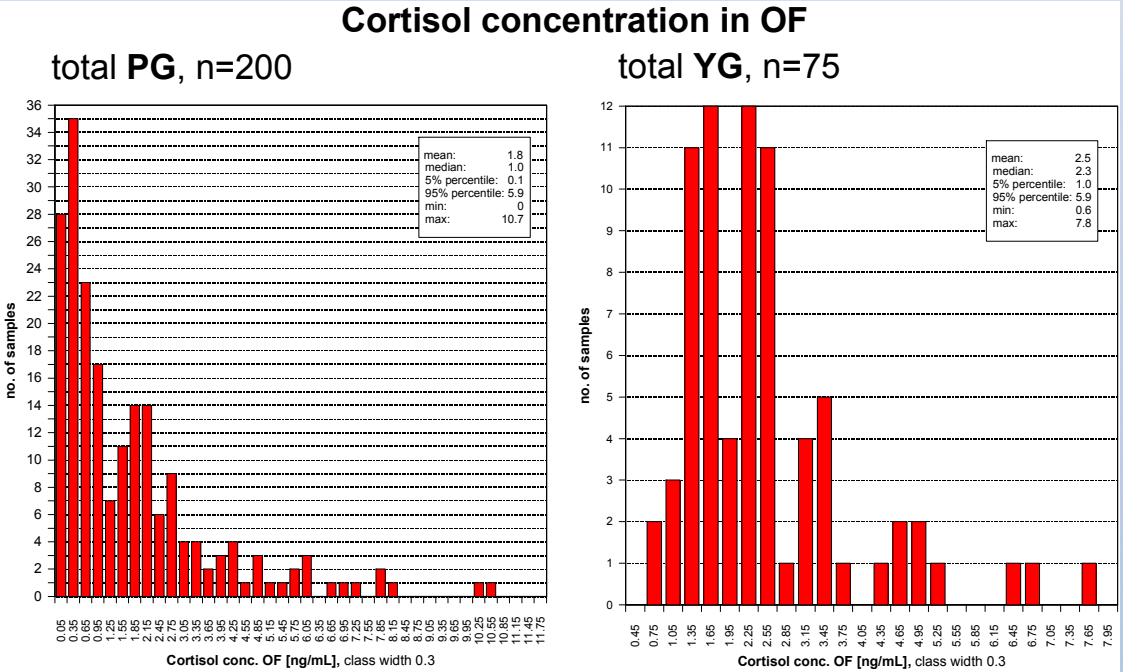
Cortisol, free
ranges during day 4 - 15 ng/ml; evening/night 0,3 - 1,5 ng/ml

“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.”

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# in saliva veritas - **Sample authenticity**

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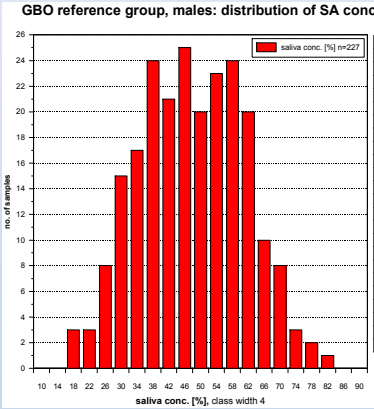
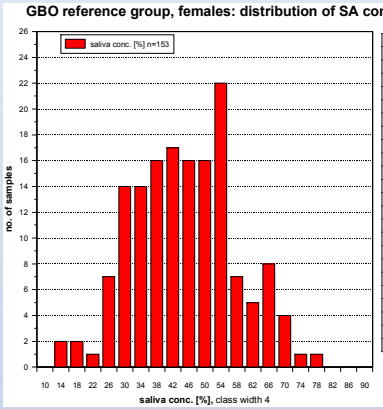
- Determination of % oral fluid
- Male: 4,15 mL -> 47,9 %



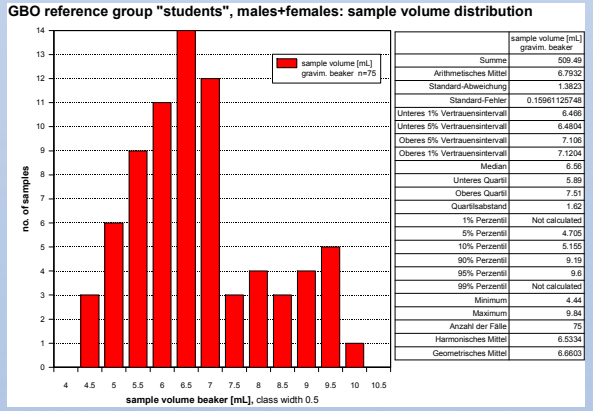

| | saliva conc. [%] |
|---|---|
| Summe | 10877.2 |
| Arithmetisches Mittel | 47.9172 |
| Standard-Abweichung | 13.1761 |
| Standard-Fehler | 0.87452591896 |
| Unteres 1% Vertrauensintervall | 46.1244 |
| Unteres 5% Vertrauensintervall | 46.2031 |
| Oberes 5% Vertrauensintervall | 49.6313 |
| Oberes 1% Vertrauensintervall | 49.71 |
| Median | 47.8 |
| Unteres Quartil | 37.7 |
| Oberes Quartil | 57.8 |
| Quartilsabstand | 20.1 |
| 1% Perzentil | 18.9 |
| 5% Perzentil | 26.4 |
| 10% Perzentil | 29.8 |
| 90% Perzentil | 65.65 |
| 95% Perzentil | 69.6 |
| 99% Perzentil | 77.65 |
| Minimum | 17.2 |
| Maximum | 83.8 |
| Anzahl der Fälle | 227 |
| Harmonisches Mittel | 43.8537 |
| Geometrisches Mittel | 45.9689 |

Female: 4,02mL -> 45 %




| | saliva conc. [%] |
|---|---|
| Summe | 6888.7 |
| Arithmetisches Mittel | 45.0242 |
| Standard-Abweichung | 12.8863 |
| Standard-Fehler | 1.0418 |
| Unteres 1% Vertrauensintervall | 42.8885 |
| Unteres 5% Vertrauensintervall | 42.9823 |
| Oberes 5% Vertrauensintervall | 47.0661 |
| Oberes 1% Vertrauensintervall | 47.1599 |
| Median | 44.7 |
| Unteres Quartil | 35.45 |
| Oberes Quartil | 53.85 |
| Quartilsabstand | 18.4 |
| 1% Perzentil | 15.75 |
| 5% Perzentil | 24.5 |
| 10% Perzentil | 28.6 |
| 90% Perzentil | 63.5 |
| 95% Perzentil | 67.25 |
| 99% Perzentil | 77 |
| Minimum | 15.6 |
| Maximum | 78.9 |
| Anzahl der Fälle | 153 |
| Harmonisches Mittel | 40.897 |
| Geometrisches Mittel | 43.0545 |

- Young group: 6,79 mL and mean 59 %




| | sample volume [mL] gravim. beaker |
|---|---|
| Summe | 509.49 |
| Arithmetisches Mittel | 6.7932 |
| Standard-Abweichung | 1.3823 |
| Standard-Fehler | 0.1596112574 |
| Unteres 1% Vertrauensintervall | 6.466 |
| Unteres 5% Vertrauensintervall | 6.4804 |
| Oberes 5% Vertrauensintervall | 7.106 |
| Oberes 1% Vertrauensintervall | 7.1204 |
| Median | 6.56 |
| Unteres Quartil | 5.89 |
| Oberes Quartil | 7.51 |
| Quartilsabstand | 1.62 |
| 1% Perzentil | Not calculated |
| 5% Perzentil | 4.705 |
| 10% Perzentil | 5.155 |
| 90% Perzentil | 9.19 |
| 95% Perzentil | 9.6 |
| 99% Perzentil | Not calculated |
| Minimum | 4.44 |
| Maximum | 9.84 |
| Anzahl der Fälle | 75 |
| Harmonisches Mittel | 6.5334 |
| Geometrisches Mittel | 6.6603 |

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# in saliva veritas - **sample authenticity**

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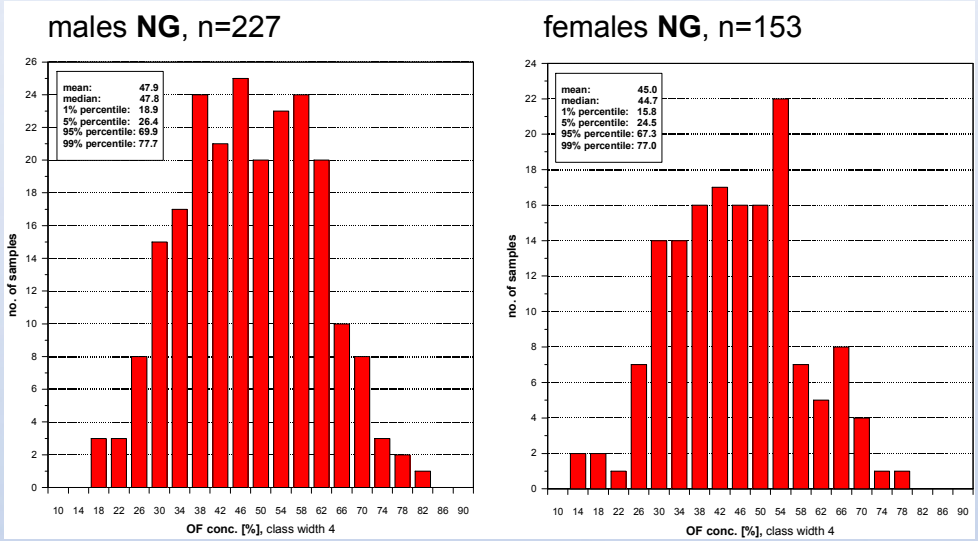
Determination of the amount of oral fluid in the collected sample




"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

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# in saliva veritas – **summary**

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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