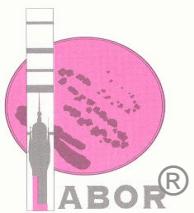




# Innovative Möglichkeiten der Drogenanalytik

Dr. Michael Böttcher

MVZ Labor Dessau GmbH



# Missbrauchsrelevante Substanzen

## Drogen (BtMG Anl. 1+2)

- Amphetamine, Methamphetamine
- Designerdrogen
- Heroin
- Cannabis
- Kokain
- LSD
- GHB
- Synthetische Cannabinoide
- .....

## Medikamente

- Methadon, Buprenorphin, Morphin
- Barbiturate
- Benzodiazepine
- Opioide
- Narkotika (Propofol, Ketamin, PCP)
- Psychopharmaka (TCA, SSRI)
- Diuretika
- Anabole Steroide

## "Naturdrogen"

- Psilocybin
- Meskalin
- "Spice"
- Muscarin
- Myristicin
- Atropin
- Scopolamin
- Kratom / Krypton
- Khat (Cathinon)

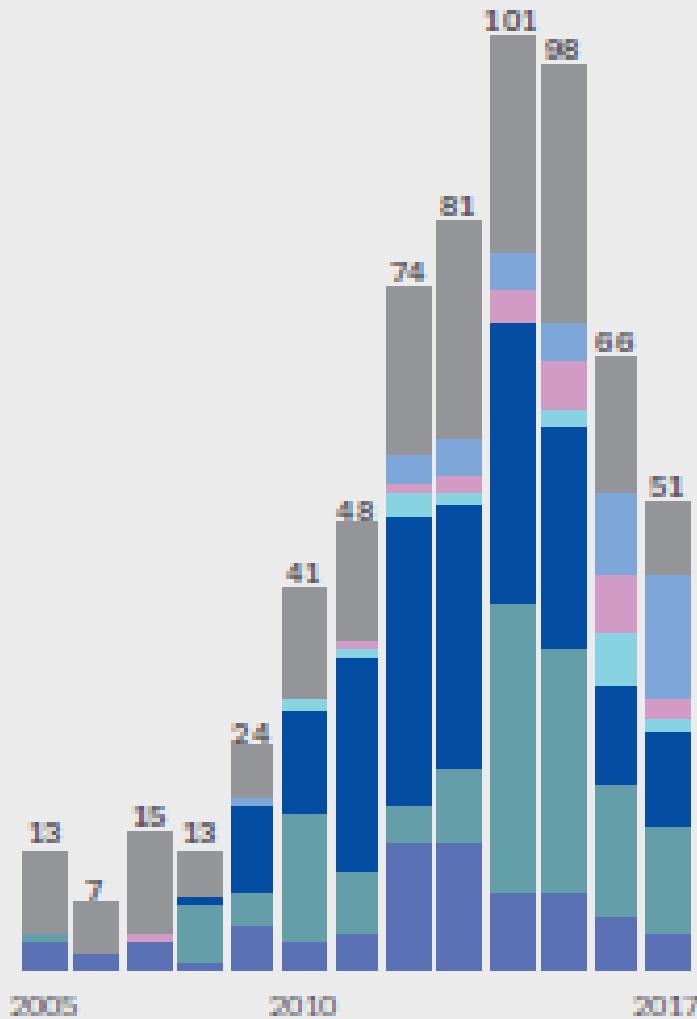
"Internetdrogen/Legal Highs/RC"  
"Badesalze"/Phenylethylamine  
"Spice"  
Tryptamine  
Opioide  
Designer Benzodiazepine

} **NPSG**

Pregabalin  
"Z-Substanzen": Zopiclon, Zolpidem,  
Zaleplon  
Methylphenidat  
Lidocain

# Neue Psychotrope Substanzen, 2009-2017

Anzahl und Kategorien der dem EU-Frühwarnsystem zuletzt gemeldeten neuen psychoaktiven Substanzen, 2005–2017



European Monitoring Center  
For Drugs and Drug Addiction,  
Lissabon  
([www.EMCDDA.europa.eu](http://www EMCDDA.europa.eu))

# **Drogenanalytik – Wie?**

## **Geschwindigkeit, Preis, Empfindlichkeit Immunoassays an Urinproben**

- Ausschluß von "Beikonsum" bei Patienten in Substitutionstherapie
- Compliance von Patienten in Substitutionstherapie (EDDP, Norbupre.)
- Ausschluß eines Drogenkonsum bei Suchtpatienten in "clean-Therapie"
  - . Einrichtungen (stationär oder ambulant)
- Ausschluß eines Drogenkonsums in JVA`s, Massregelvollzug
- "workplace testing", Arbeitsmedizin
- psychiatrische (Sucht-)Kliniken
- Forensik, Rechtsmedizin

# Welche Immunoassays (Urin!) gibt es?

- Amphetamine and derivatives (!?)
  - Barbiturates
  - Benzodiazepines
  - Cocaine (Benzoylecgonine)
  - Methadone or better EDDP
  - Opiates
  - 6-Monoacetylmorphine
  - Cannabinoids (THC-COOH)
  - Tramadol
  - Oxycodone
  - Buprenorphine
  - Fentanyl
  - "Spice,, 2x
  - LSD
  - Ethanol
  - Phencyclidine
  - Propoxyphene
  - Methaqualone
  - Tricyclic Antidepr.
  - Paracetamol
  - Salicylates
  - Ethylglucuronide
- 
- neu: Pregabalin

# Drugs of abuse screening - urine

## Immunoassay (CEDIA)

Amphetamines  
Barbiturates  
**Benzodiazepines**  
Cocaine  
Methadone/EDDP  
Opiates  
**THC-COOH**

500  
100  
100  
50  
100  
100  
25

0.5 LSD  
5.0 6-MAM  
2.0 Bupren.

target screening  
"1 ng/mL"

serum  
postm. blood  
oral fluid  
gastric content  
hair  
capillary blood  
meconium

confirmation or extended screening  
GC/MS, GC/MS-MS or LC/MS-MS  
after dedicated sample preparation

GC/MS "general unknown"  
enzym. hydrolysis+ LLE+acetylation

Amphetamines      Designer drugs

Methadone  
Opiates

Analgesics (Tilidine, Oxycodone, etc.)  
Antidepressants

Antiepileptics

Neuroleptics

NSAIDs

Zopiclone, Zolpidem, Zotepine

:

:

:

:

:

etc.

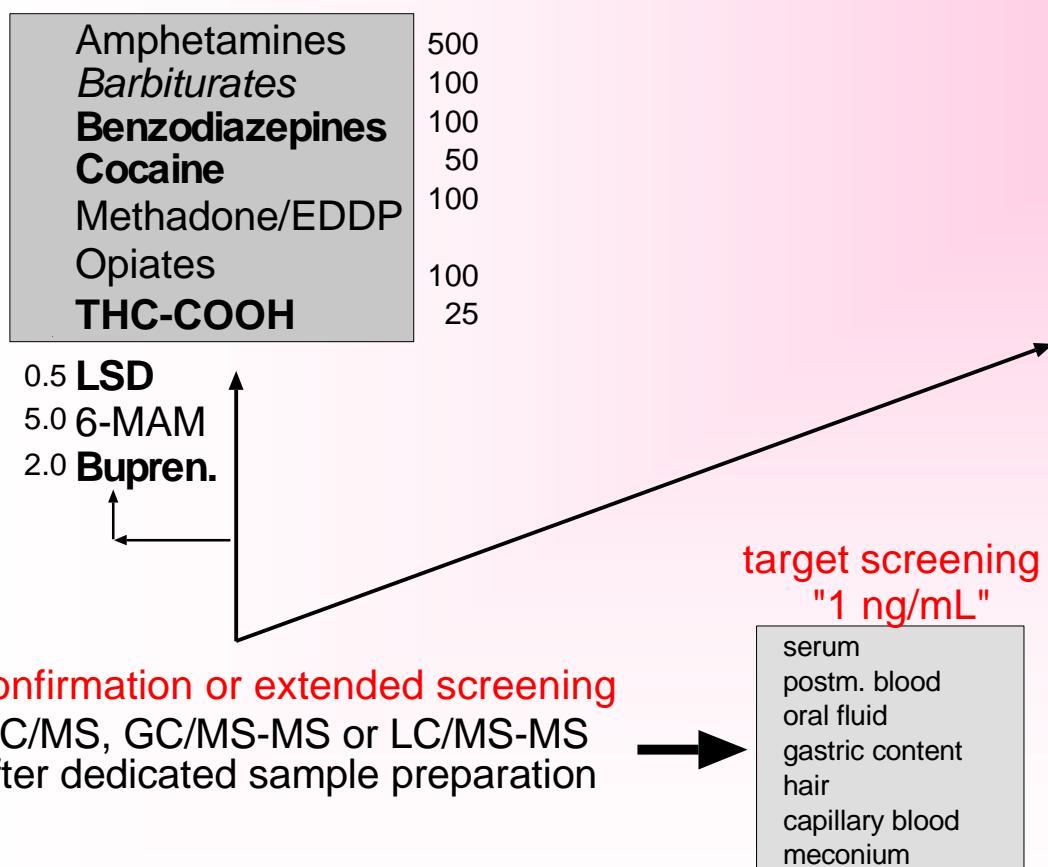
broad screening, "100 ng/mL"

# Drogenscreening (mit Immunoassay) im Urin -- Probleme:

- in vivo/vitro Verdünnung! Kreatinin korrigierter Cutoff?!
- pH-Wert: 4.5 bis 9.0!! pH-abhängige Resorption, Stabilität
- Manipulation der Probe: Verdünnung, Detergenzien etc., Oxidantien, Abgabe „Freundurin“, Zugabe d. Medikamente: Probennahme unter Aufsicht
- Cutoffs: insbes. Gruppenteste nicht standardisiert
- Kreuzreaktivität: falsch positiv / **falsch negativ**
- Zunehmende Zahl von Drogen, neue **Substanzklassen**, unbekannte Metaboliten
  - **andere Matrix** (Muttersubstanzen)
  - **andere** (sensitivere) **Methoden**

# Drugs of abuse screening - urine

## Immunoassay (CEDIA)



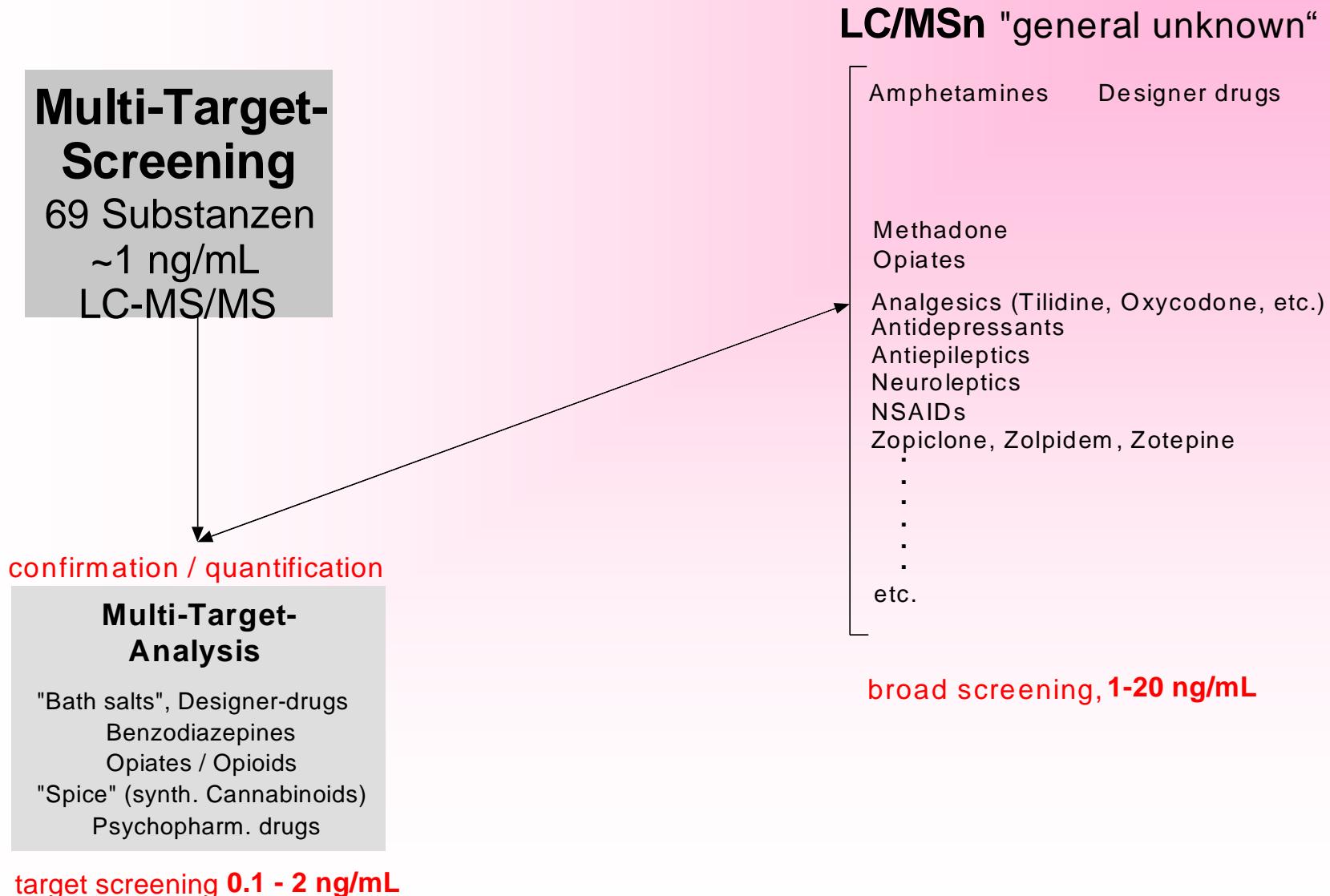
GC/MS "general unknown"  
enzym. hydrolysis+ LLE+acetylation

Amphetamines      Designer drugs  
  
Methadone  
Opiates  
  
Analgesics (Tilidine, Oxycodone, etc.)  
Antidepressants  
Antiepileptics  
Neuroleptics  
NSAIDs  
Zopiclone, Zolpidem, Zotepine

etc.  
etc.  
etc.  
etc.  
etc.

broad screening, "100 ng/mL"

# Drugs screening - **serum**, meconium, hair, **oral fluid**, etc.



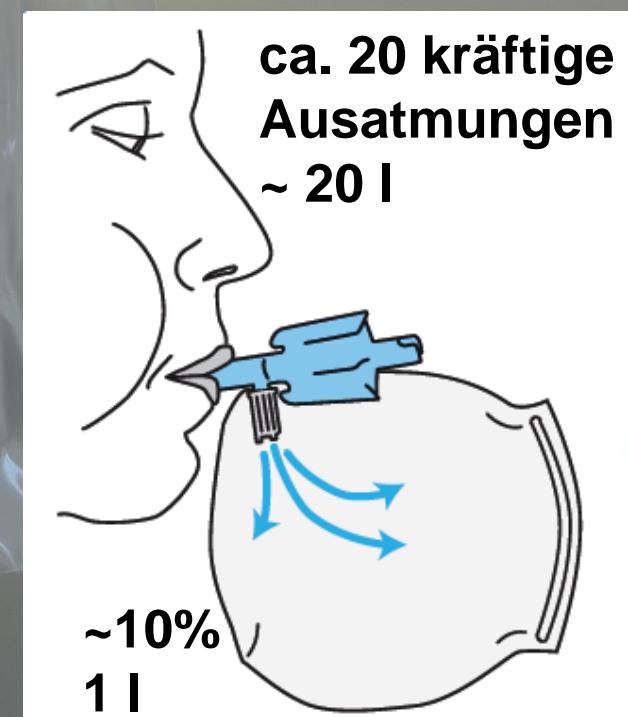
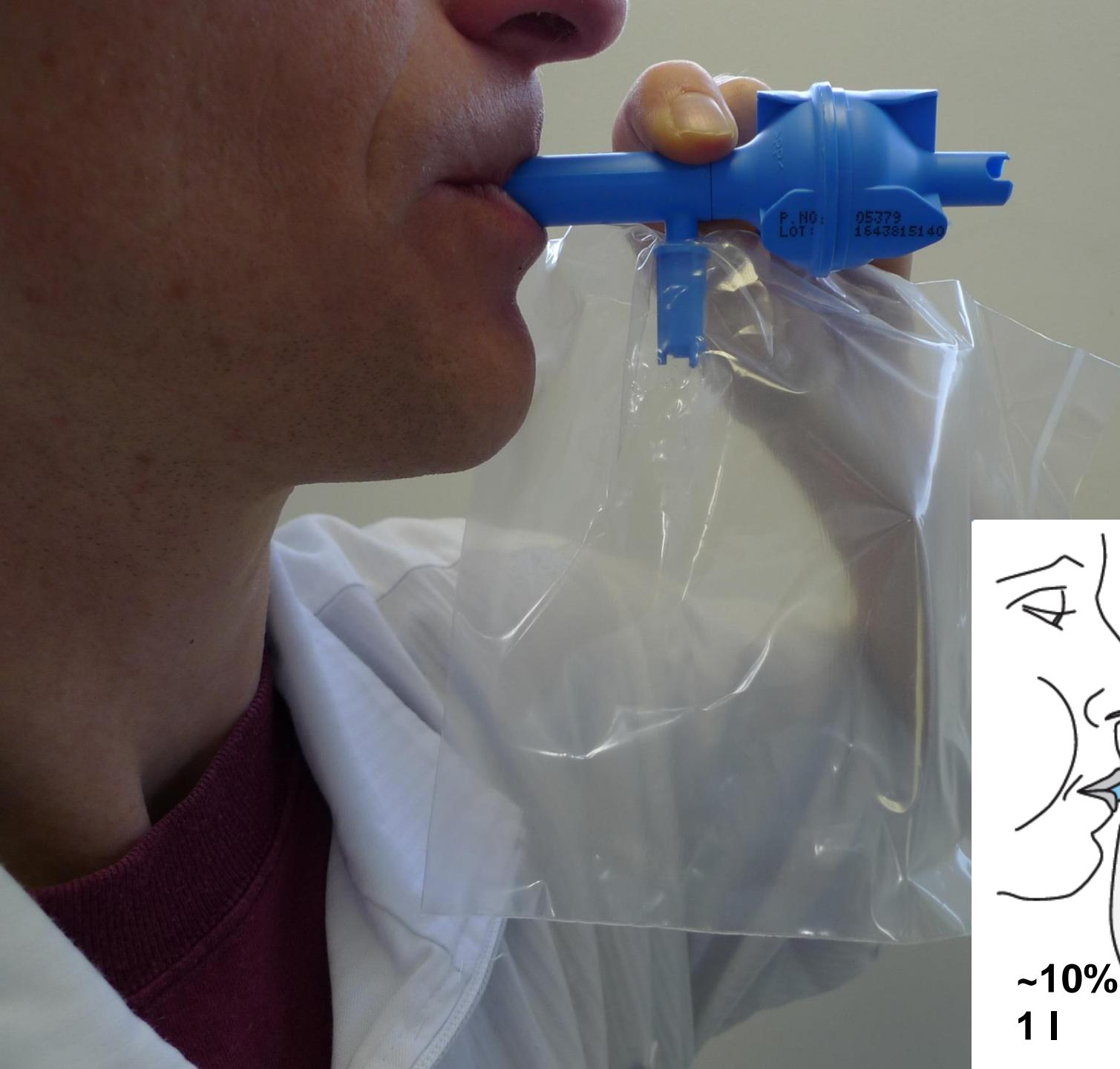
Nicht invasive Probennahmen, Nachw.fenster 1-7 d:  
1. Kapillarblut, 2. Speichel („Oral Fluid“), 3. Ausatemluft

- **Multi-Target-Screening, 69 Subst.**  
wichtigste Vertreter der verschiedenen Substanzklassen: Amph, Benzo, Kokain, Opiate/Opiode, THC, Substitute
- **Multi-Target-Analysen**  
(voll-) umfängliche Analytik der versch. Substanzklassen: „Spice“, Opiate/Opiode, Amph+„Badesalze“, Benzo, Psychopharmaka

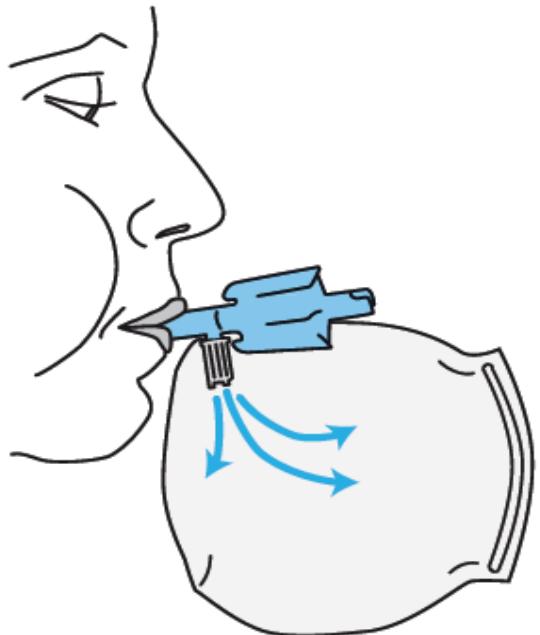
# Drogen in der Ausatemluft?



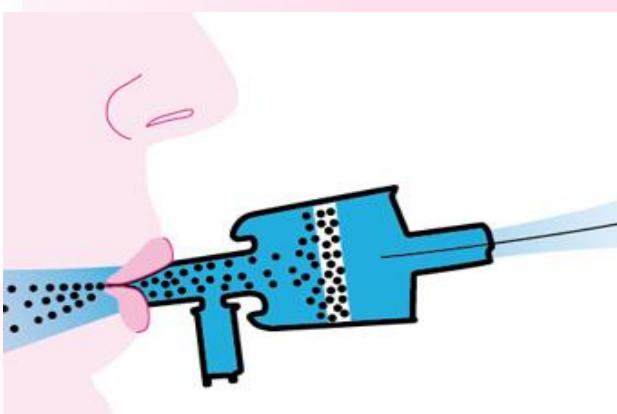
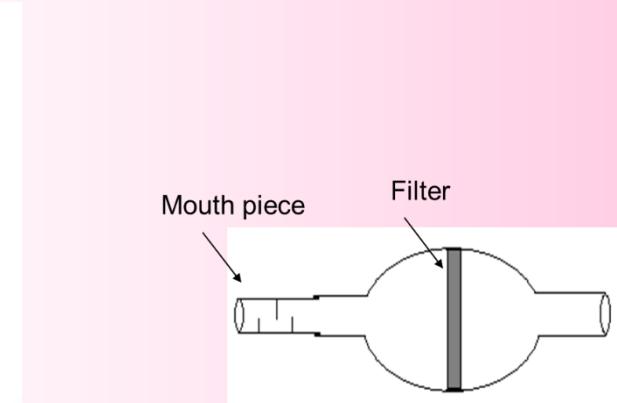
Sind Drogen flüchtig.....???  
.....



# Sammelsystem für die Aerosol Partikel in der Ausatemluft



2-3 min



Aerosol particles



**2010**

# **1. Publikation**

Journal of Analytical Toxicology, Vol. 34, June 2010



## **Amphetamines Detected in Exhaled Breath from Drug Addicts: A New Possible Method for Drugs-of-Abuse Testing**

**Olof Beck<sup>1,\*</sup>, Kathinka Leine<sup>1</sup>, Göran Palmskog<sup>1</sup>, and Johan Franck<sup>2</sup>**

<sup>1</sup>*Department of Medicine, Section of Clinical Pharmacology* and <sup>2</sup>*Department of Clinical Neuroscience, Division of Psychiatry,  
Karolinska Institutet, Stockholm, Sweden*

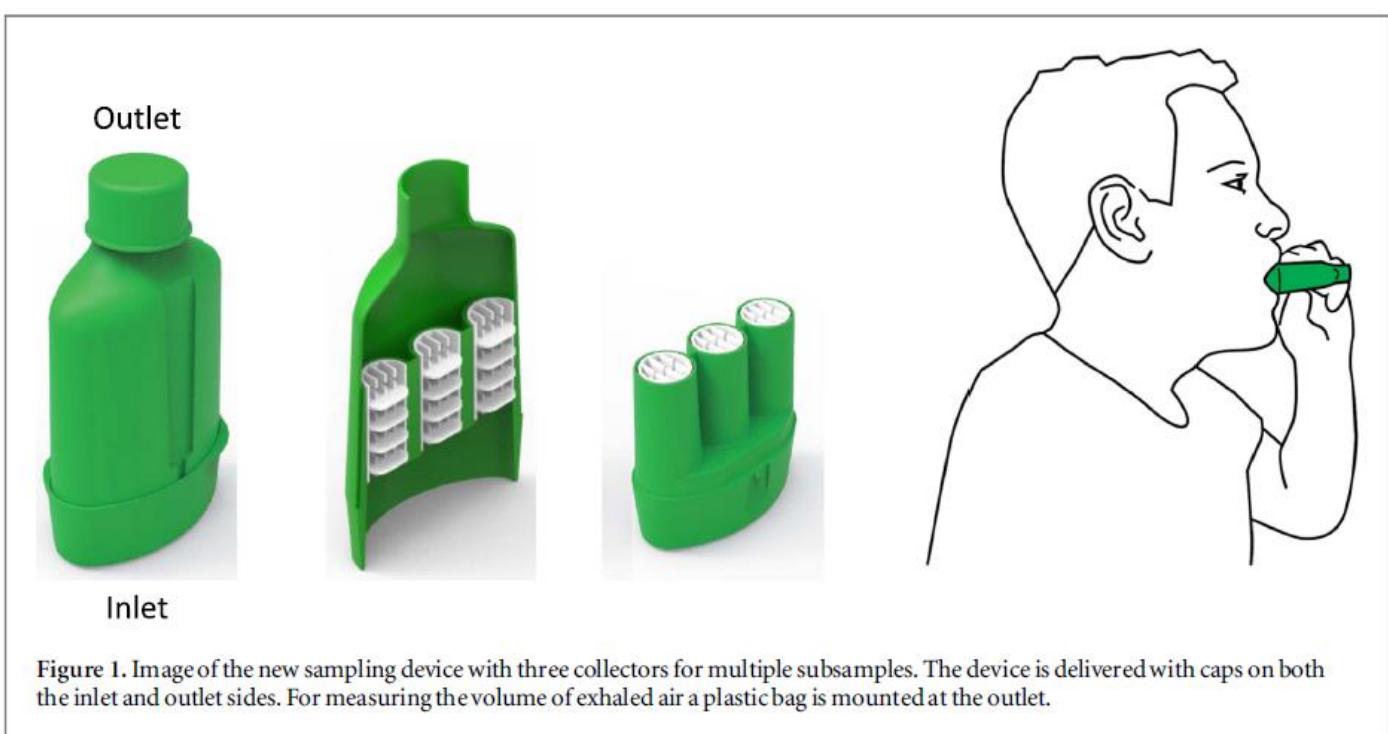
**Conclusion:** Drug intake can be detected in breath after sampling on filter and LC-MS/MS analysis

# Neues verbessertes Sammelsystem

PAPER

Evaluation of a new simple collection device for sampling of microparticles in exhaled breath

Sabina Seferaj<sup>1</sup>, Shahid Ullah<sup>1,2</sup>, Åsa Tinglev<sup>2</sup>, Sten Carlsson<sup>3</sup>, Jesper Winberg<sup>4</sup>, Peter Stambeck<sup>4</sup> and Olof Beck<sup>1,2</sup> 





Contents lists available at ScienceDirect



## Sports drug testing using complementary matrices: Advantages and limitations



Mario Thevis<sup>a,b,\*</sup>, Hans Geyer<sup>a,b</sup>, Laura Tretzel<sup>a</sup>, Wilhelm Schänzer<sup>a</sup>



### **RESEARCH ARTICLE**

# Expanding analytical options in sports drug testing: Mass spectrometric detection of prohibited substances in exhaled breath

Mario Thevis<sup>1,2</sup> | Oliver Krug<sup>1,2</sup> | Hans Geyer<sup>1,2</sup> | Wilhelm Schänzer<sup>1</sup>

Besides stimulants such as methylhexaneamine and pseudoephedrine, also the anabolic-androgenic steroid dehydrochloromethyltestosterone, the metabolic modulator meldonium, and the beta-blocker bisoprolol was detected in exhaled breath.

**Conclusions:** The EB aerosol has provided a promising proof-of-concept suggesting the expansion of this testing strategy as a complement to currently utilized sports drug testing programs.

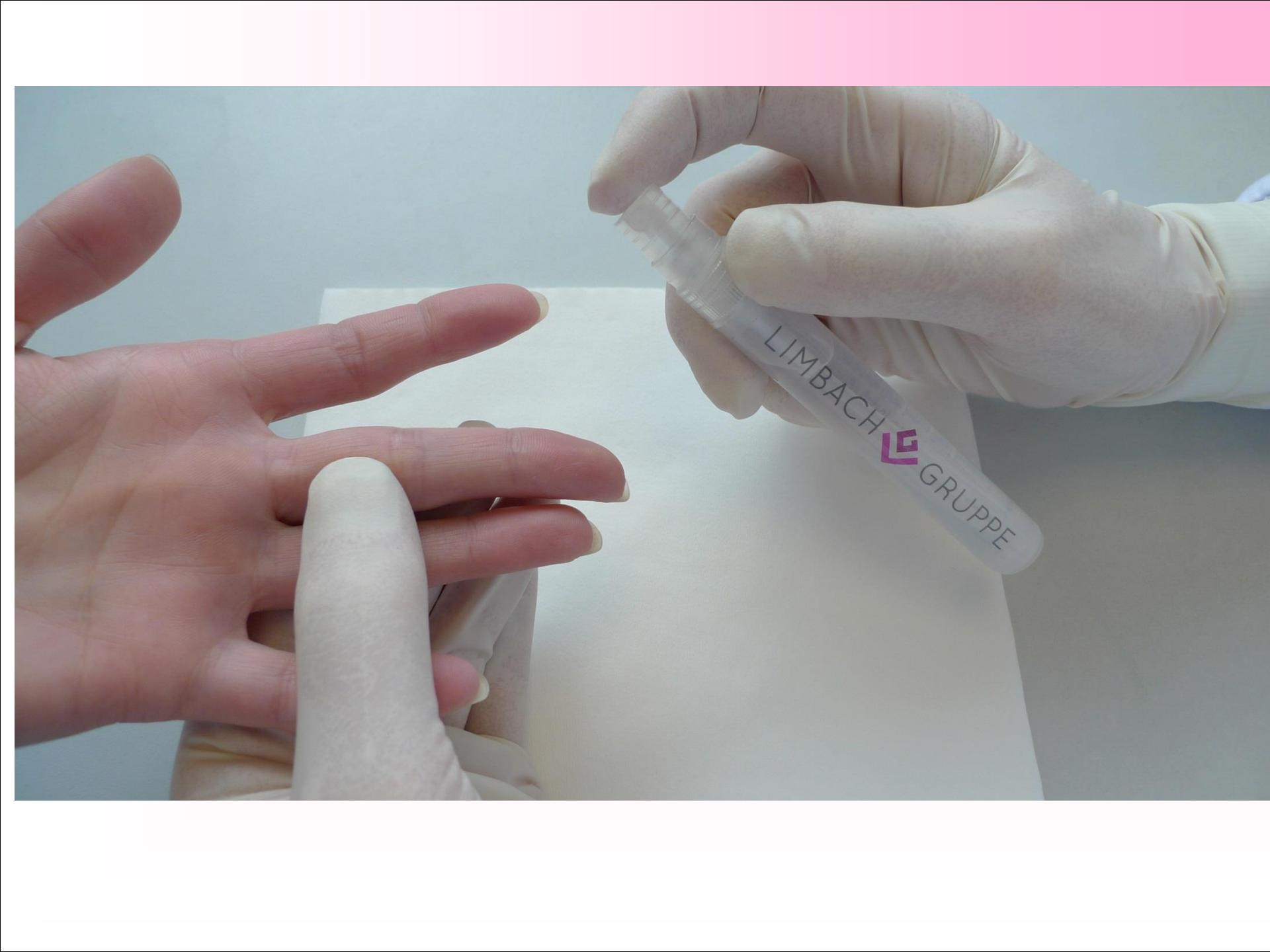
# Kapillarblut



EDTA-Blut  
20 µL

# Wärmekissen 50°C

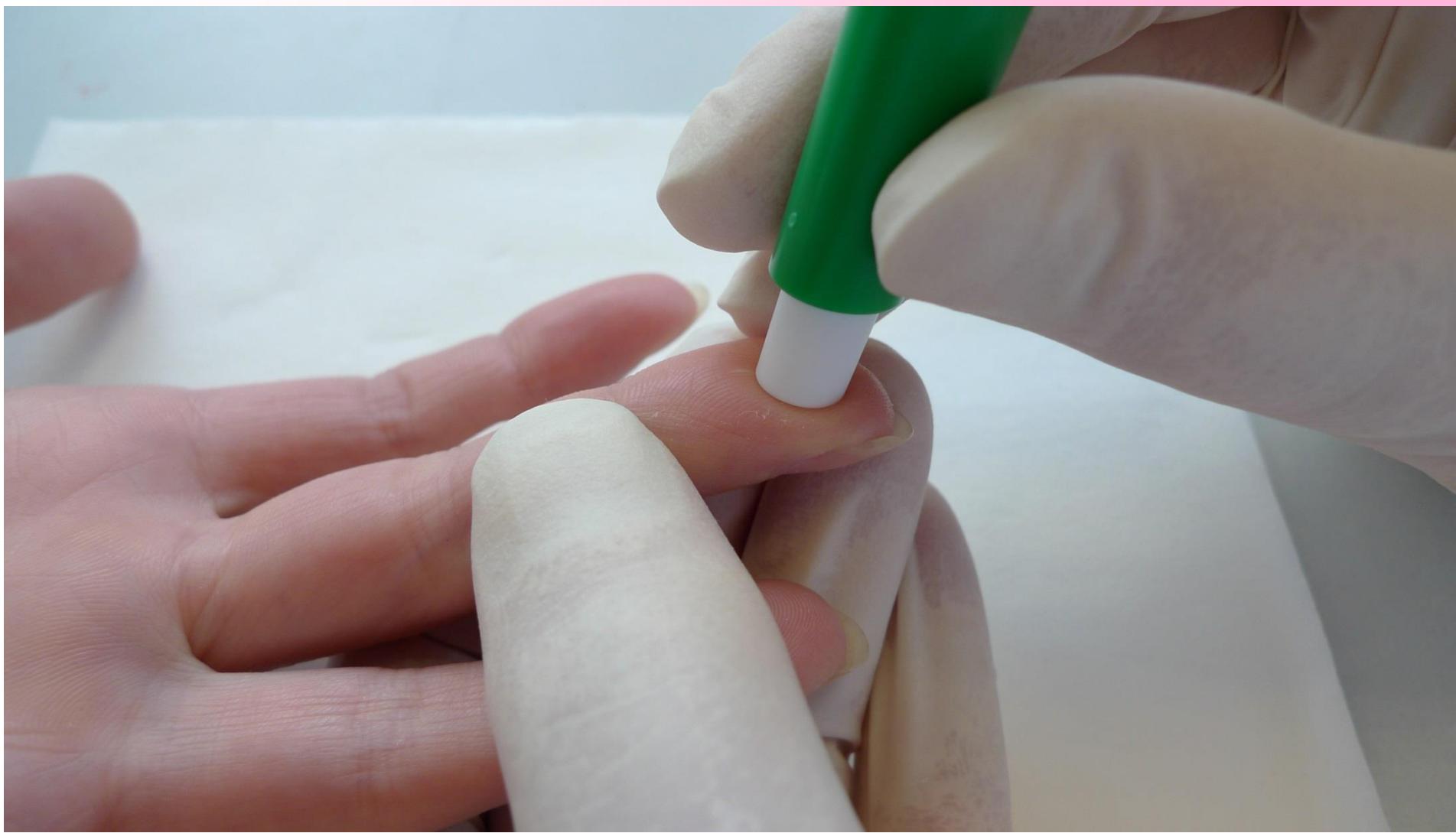




LIMBACH



GRUPPE





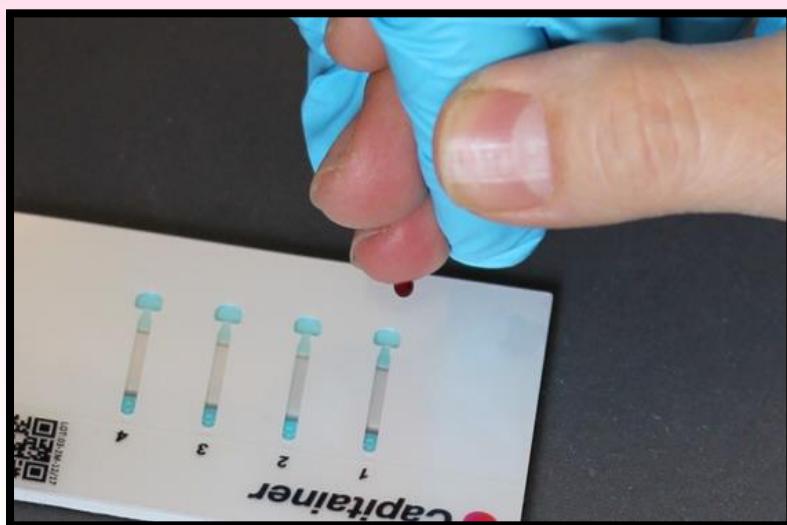
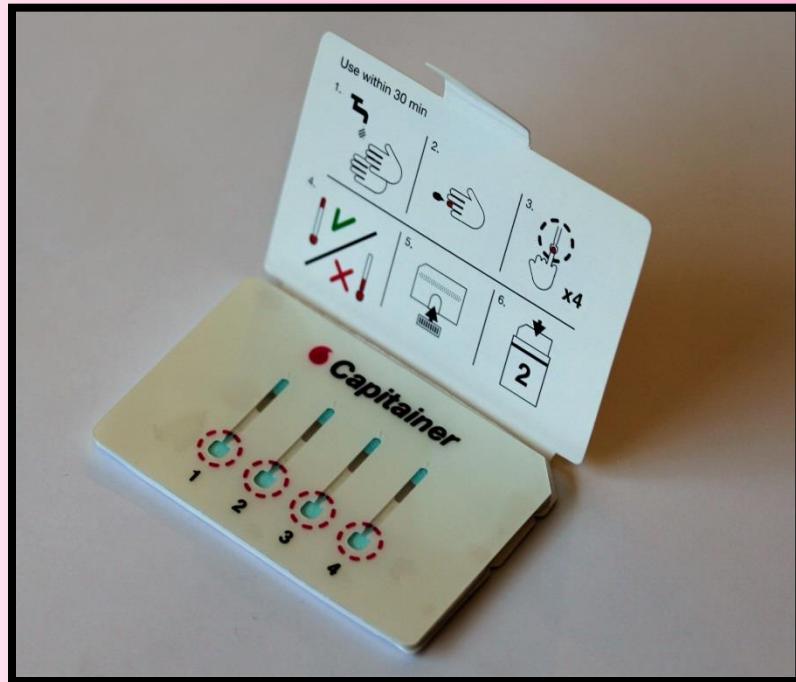




- Oft nur 1 Probe
- Volumen 20  $\mu\text{L}$ ?

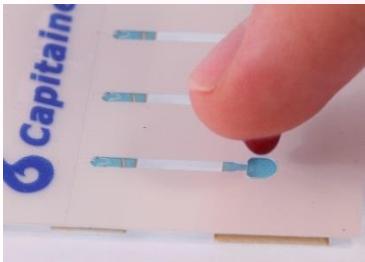


# Capitainer



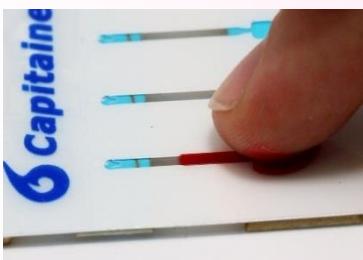
# Sammelprozedur

1



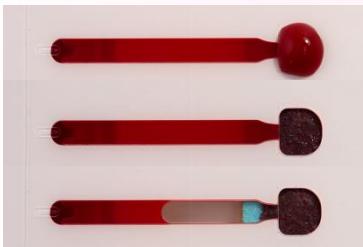
Apply a drop of blood to each inlet port of the Capitainer DBS card.

2

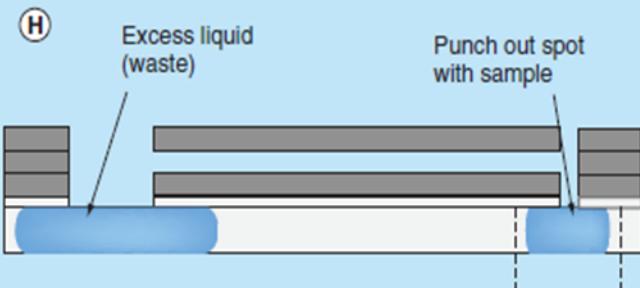
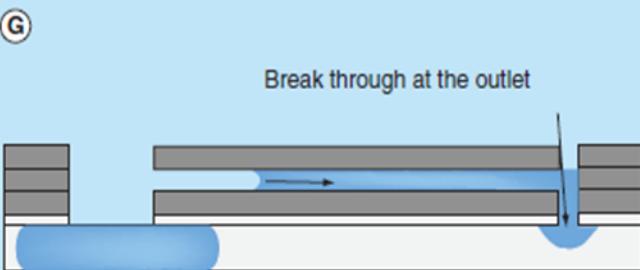
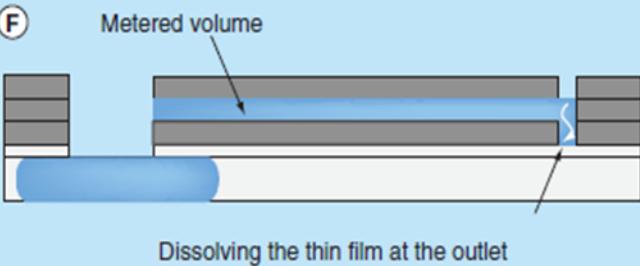
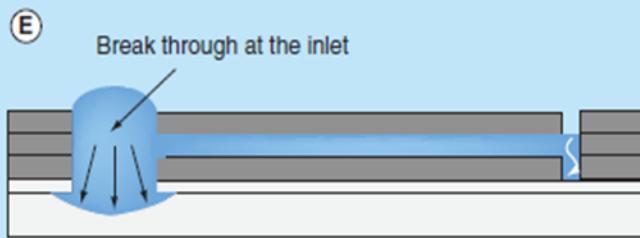
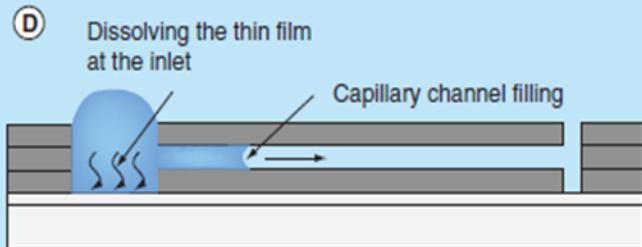
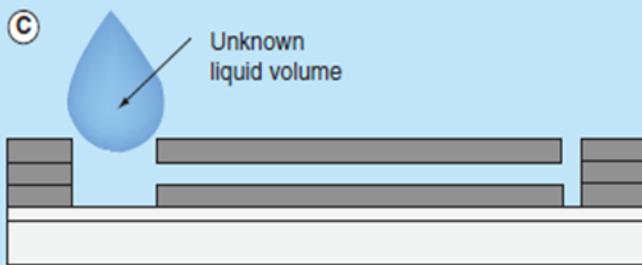
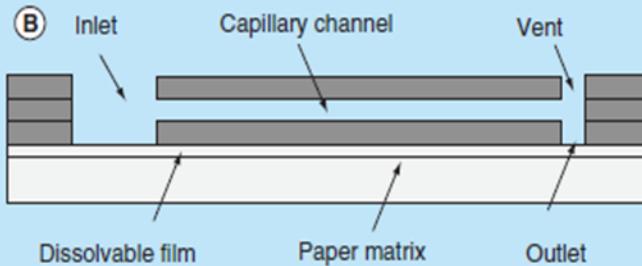
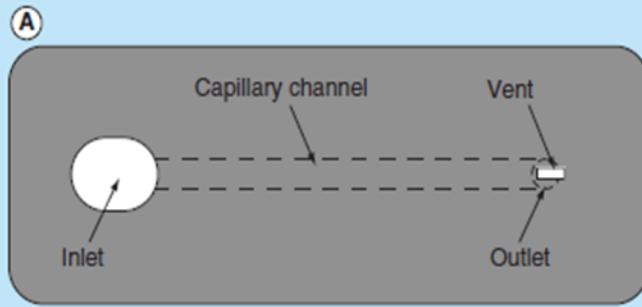


Touch the inlet port with the drop and transfer at least 30 µl to the card.

3



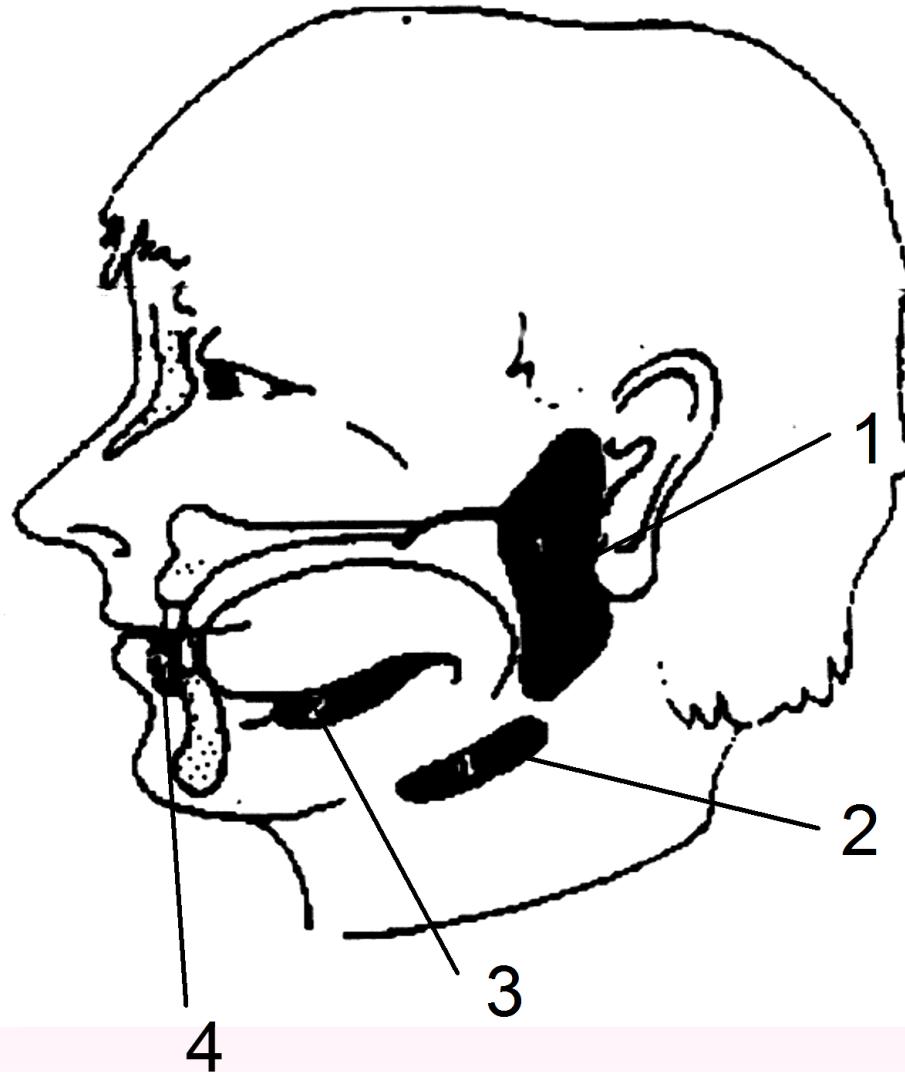
Automatic channel filling, removing of excessive blood and DBS formation.



# Im Labor.....



# Speichel



## Bezeichnung der Speicheldrüsen

1 paarige Ohrspeicheldrüse (*Glandula parotis*), 2 Unterkieferdrüse (*Gl.submandibular*),  
3 Unterzungendrüse (*Gl.sublingualis*), 4 Lippendrüsen (*Gl.labiales*) [4]

*Parotisspeichel*: dünnflüssig, nicht fadenziehend

*Submandibularisspeichel*: klar, schleim ähnlich

*Mundschleim*: dick, sehr zäh, fadenziehend

*Gemischter Speichel*: ein wenig fadenziehend, von geringer Viskosität.

# **Wissenswertes zum Speichel**

- Produktion: bis 1.5 L/d, Fluss: 0.3-0.5 / 1.0-1.5 mL/min
- Parotis(1) ~20%, Submandibularis(2) ~70%, Sublingualis(3) ~5%
- Ruhesekretion: **pH 5.8 - 6.4**, Stimulation: bis 7.8
- "Zutaten":
  - H<sub>2</sub>O 99%
  - Enzyme: **Amylase**
  - Mucine
  - IgA u. a. Ig
  - Elektrolyte: Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, HCO<sub>3</sub><sup>-</sup>
  - bei niedrigem Fluss: hypoton
  - bei hohem Fluss: isoton

# Wie gelangen Drogen in den Speichel?

-- Orale Kontamination

-- aus dem Blut durch **passive Diffusion**

-- aktive Sekretion

-- Filtration



Faktoren mit Einfluß auf das S/P-Ratio:

- **pKa** einer Substanz (sauer - alkalisch?)
- Lipidlöslichkeit
- **Plasmaprotein Bindung**
- Molekulargewicht

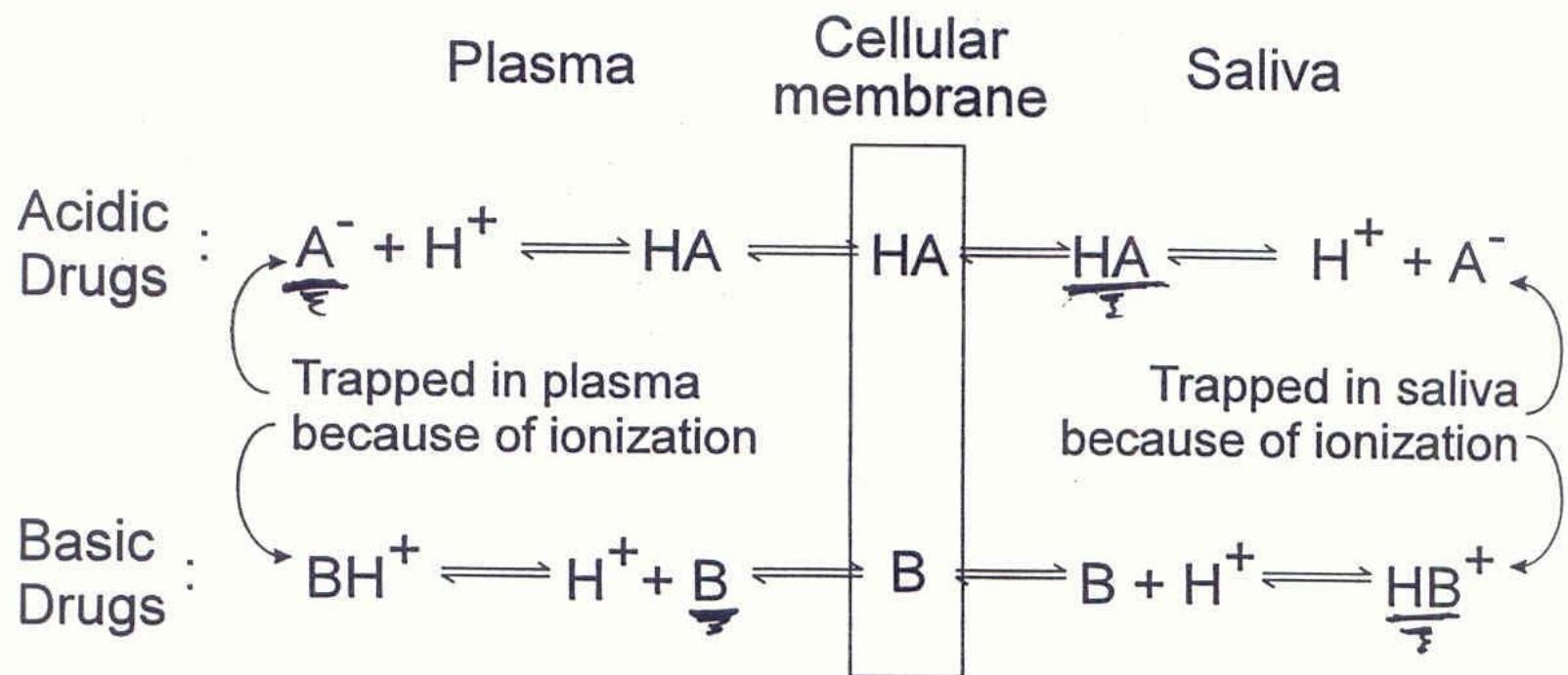
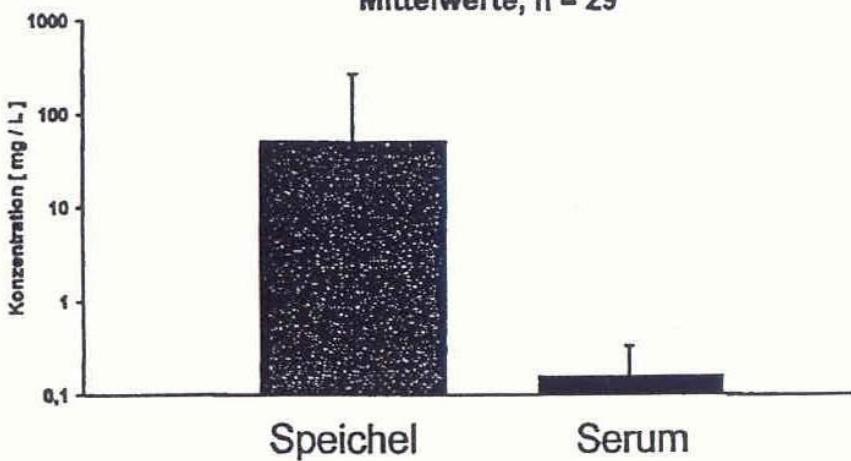
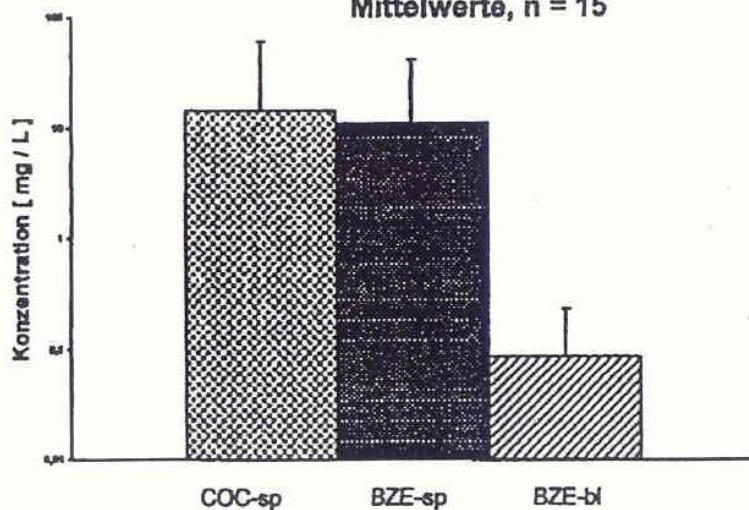


Fig. 1. Schematic diagram for transport of drugs into saliva or sweat.

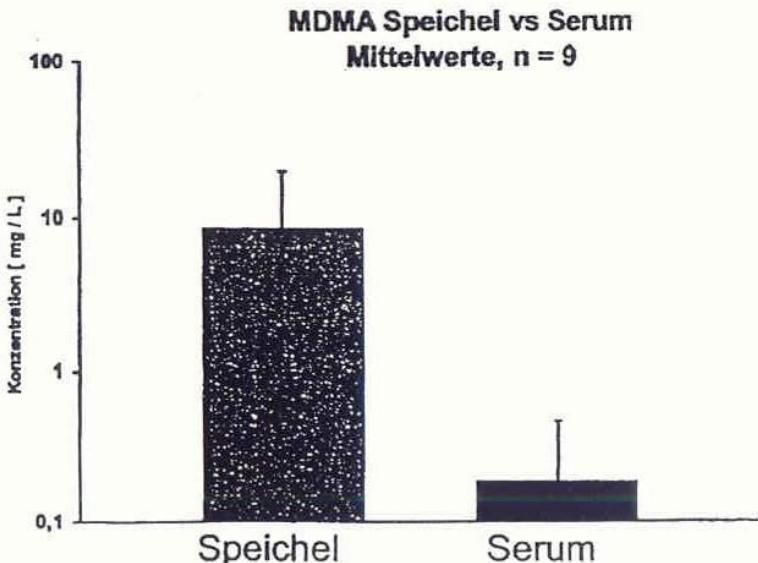
**Amphetamin Speichel vs Serum**  
Mittelwerte, n = 29



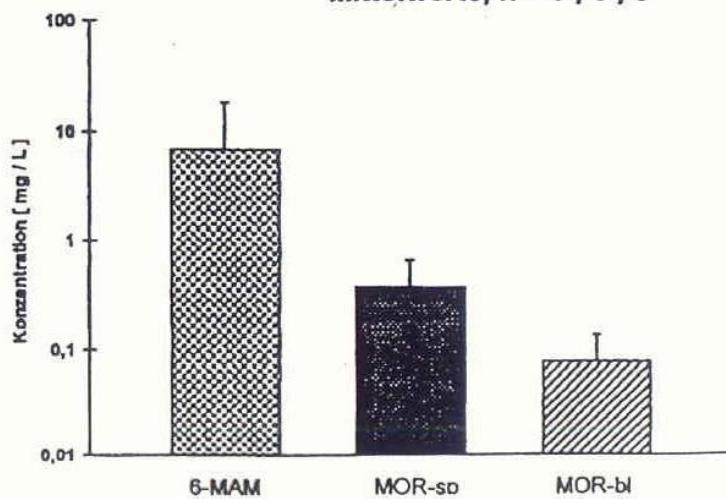
**Cocain / BZE Speichel vs Serum**  
Mittelwerte, n = 15



**MDMA Speichel vs Serum**  
Mittelwerte, n = 9



**Opiate Speichel vs Serum**  
Mittelwerte, n = 3 ; 6 ; 9



# Saliva Collection System (SCS) pH 4.2

## Greiner Bio-One

### 4 ml Saliva Extraction Solution (SES)

contains non-toxic yellow  
food color and buffer salts



Saliva Collection Beaker  
with integrated saliva transfer  
device

2



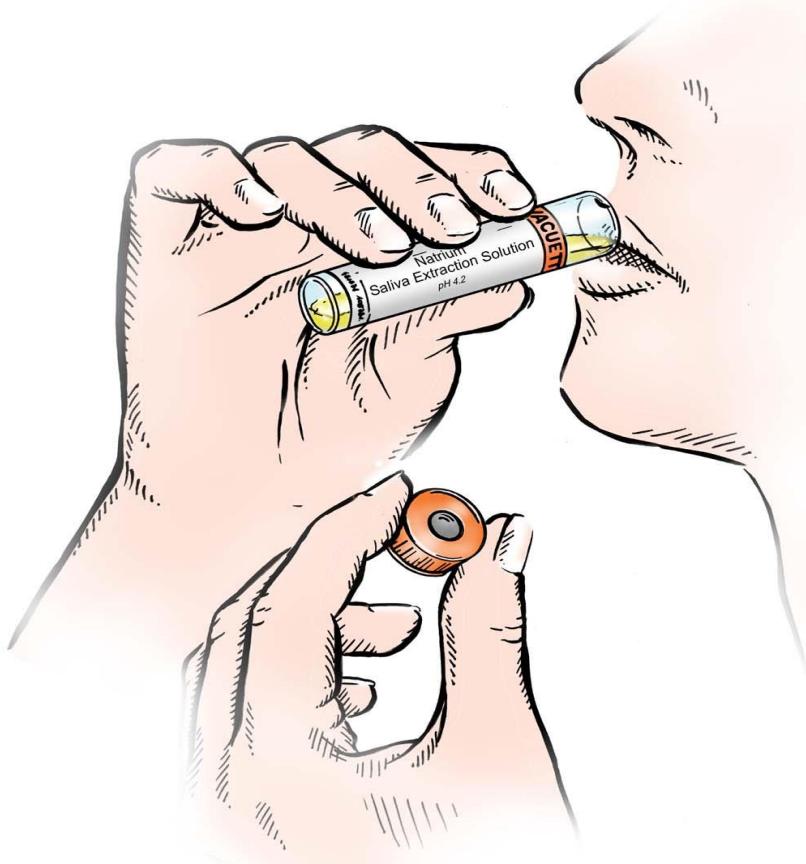
1

3  
a  
b

Evacuated Saliva  
transfer tubes  
contains stabilizing  
agents ; A+B sample!

# OF sampling with the Greiner Saliva Collection System:

## Step 1



**Rinsing of the oral cavity with  
Saliva Extraction Solution for  
2 minutes**

**Step 2:**



**Spitting of the extracted  
oral fluid into the Saliva  
Collection Beaker**

### Step 3



**Transferring of the extracted OF  
into the evacuated  
Saliva Collection Tubes**

**%OF is determined by  
photometry on Olympus AU680  
always A + B sample!**

#### **Advantages:**

- quick (Xerostomia!), standardized time
- acidic pH during collection keeps pH difference to plasma
- acidic pH: 6-AM, Cocaine, Zopiclone etc. are stable
- aqueous matrix: less ion suppression, rapid SALLE possible

# Perianalytik

Perianalytik bezeichnet Überprüfung der Probe vor der eigentlichen Analytik (Echtheit, Verdünnung, Manipulationen etc.)

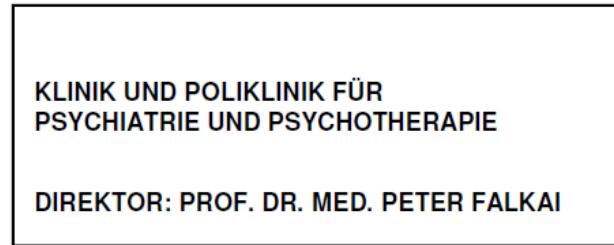
1. Probenvolumen
  2. Photometrische Bestimmung des Speichelanteils (optische Dichte des Tartrazins)
  3. Photometrische Bestimmung der Alpha-Amylase Konzentration durch die Messung der Alpha-Amylase Aktivität (1. Authentizitätsmarker)
  4. Bestimmung der Cortisolkonzentration (2. Authentiziätsmarker) mit UPLC-MS/MS
- Böttcher M., Preidel A., Beck O. “Peri-analytics” reference ranges for drug screening in oral fluid using the Greiner Bio-One collection device”; Poster: TIAFT, Bonn (2010)

# Routine Drogenscreening im Speichel mit UPLC-MS/MS: Analyten

Analytes in „**Module A**“, cutoff 0.1-2 ng/mL neat OF, IS = 0.5 ng/mL SA/SES:

- **Peri-analytics:** volume, % saliva in SES, Amylase (Roche AU680), Cortisol (incl. in LC-MS/MS)
- **Substitution drugs:** D-/L-Methadone, EDDP, Buprenorphine, Norbuprenorphine
- **Amphetamines:** Amphetamine, Methamphetamine, MDMA, MDA, MBDB, BDB, MDEA, PMMA, Butylone, Mephedrone, Methylone, MDPV, alpha-PVP, 4-Methylethcatinon, Pentedron
- **Benzodiazepines:** Diazepam, Nordiazepam, Oxazepam, Midazolam, Flurazepam, Desalkyl-flurazepam, Temazepam, 7-Aminoclonazepam, Alprazolam, Flunitrazepam, 7-Aminofluni-trazepam, Bromazepam, Lorazepam, Phenazepam
- **Cocaine:** Cocaine, Benzoylecgonine, Methylecgonine, Lidocaine
- **Opiates:** Morphine, Codeine, 6-Acetylmorphine, 6-Acetylcodeine, Norcodeine, Dihydrocodeine
- **Opioids:** Naloxone, Tilidine, Tramadol, O-Desmethyltramadol, Oxycodone, Noroxycodone, Fentanyl, Nortilidine, Hydromorphone, Noscapine, Loperamide, Dextromethorphan
- **Cannabinoids:** THC
- **Others:** Zolpidem, Zopiclone, Zaleplone, Ketamine, Methylphenidate, Ritalinic acid, Pregabalin, Gabapentin, Bupropion, Diphenhydramin, Mirtazapine, Quetiapine, Doxepin

**actual: N = 69 (3 transitions) + 64 deuterated IS (2 transitions)**



## Nachweis von Pregabalin in der Substitutionstherapie opiatabhängiger Patienten im Rahmen eines Multi- Targetscreenings im Speichel

Martin G<sup>1</sup>, Böttcher M<sup>2</sup>, Walcher S<sup>3</sup>, Musselmann R<sup>3</sup>, Plörer D<sup>1</sup>, Winter C<sup>1</sup>, Canolli M<sup>1</sup>, Pogarell O<sup>1</sup>

<sup>1</sup>Klinik für Psychiatrie und Psychotherapie der LMU München, <sup>2</sup>MVZ Labor Dessau GmbH,  
Dessau-Roßlau, <sup>3</sup>Schwerpunktpraxis Concept, München

	Patienten	Proben	Patienten Preg. pos.	Patienten BZD pos.	
Gesamt	389	787	90	75	23% / 19%
N5	134	532	44	38	33% / 28%
Concept	255	255	46	37	18% / 15%

223 samples Pregabalin positive = 28%

## **Nord-Süd Vergleich Raum Berlin, 2015**

- 1000 Urinproben von Patienten in Substitutionstherapie
- Zufällig/anonymisiert ausgewählt aus Routineanalytik
- 752 m, 248 f
- **125 Proben positiv ( $>10 \text{ ng/mL}$ ) = 12.5%**

Jungen  
in hohen  
Tosten  
gefährden  
Ihre  
Gesundheit!



**Vielen Dank für Ihre  
Aufmerksamkeit!**

[michael.boettcher@laborpraxis-dessau.de](mailto:michael.boettcher@laborpraxis-dessau.de)