Oral fluid as an alternative matrix for drugs of abuse testing

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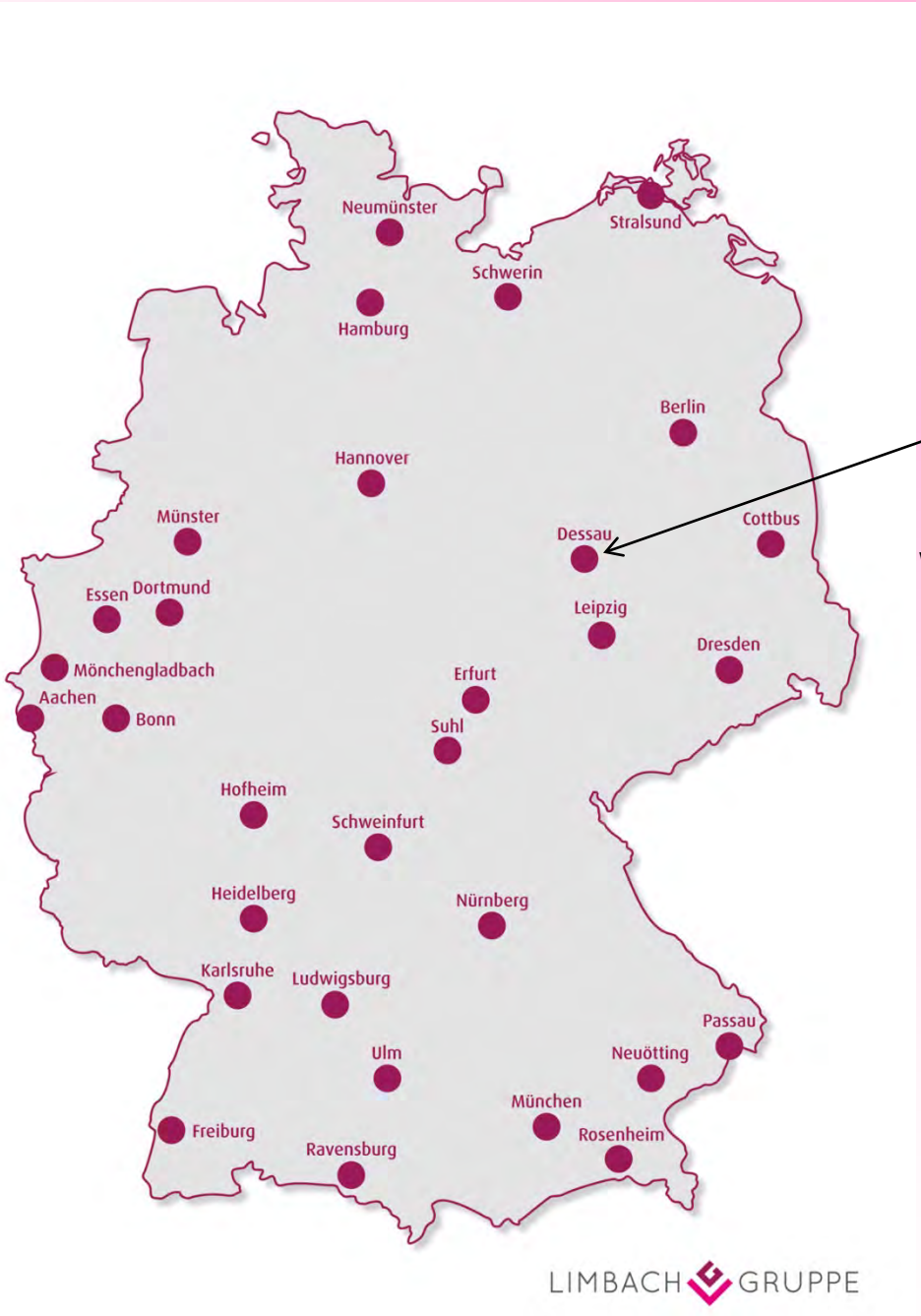

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Drugs of abuse - who?

Patient
- physicians, GP
- (outpatient) substitution therapy
- rehabilitation-, addiction-hospitals
- other therapeutic settings
- hospitals (intoxications)

"Subject/person"
- prisons
- health authorities
- occupational medicine
- workplace testing
- unemployment agencies
- traffic/driving license authorities
- police

Forensic

Athlets/sports

Which immunoassays (urine) are available?

-- Amphetamin and derivatives (!?)
-- Barbiturates
-- Benzodiazepines
-- Cocaine (Benzoylecgonine)
-- Methadone or better EDDP
-- Opiates
-- 6-Monoacetylmorphine
-- Cannabinoids (THC-COOH)
-- Tramadol
-- Oxycodone
-- Buprenorphine
-- Fentanyl
-- "Spice„ 2x

-- LSD

-- Phencyclidine
-- Propoxyphene
-- Methaqualone

-- Tricyclic Antidepr.
-- Paracetamol
-- Salicylates

-- Ethylglucuronide

-- Ethanol

Trias: speed (POCT!) , price, sensitivity

Problems in drugs of abuse testing:

-- new substances, urine EIAs do not cover
-- false negatives / false positives with EIA
-- cutoffs not standardized (esp. group test EIAs)

Urine:
-- diuresis! Creatinine dependent cutoff needed!
-- authenticity/adulteration: supervised sampling needed
-- metabolites not available for many new drugs
-- difficult matrix for LCMS

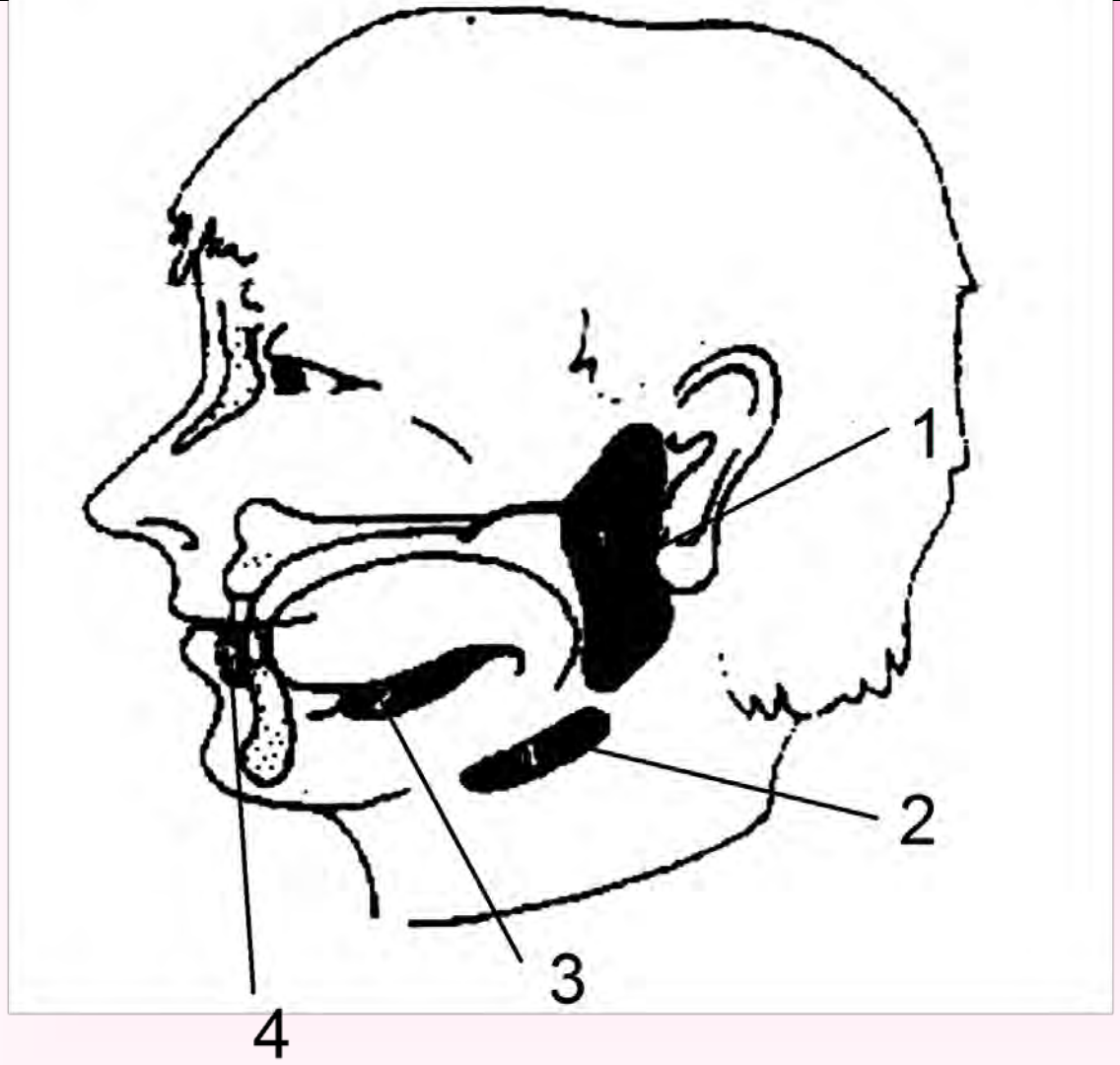
Saliva (Oral Fluid)
-- no dilution problems but sampling problem (which device)?
-- easy supervision of sampling
-- mostly parent drugs needed!, easier method devlopment!(?)
-- „cleaner“ matrix: easier method development

but: which analytes in OF at which concentration?

- decided for a liquid-based collection device – why?
- developed a sensitive UPLC-MS/MS method for the detection of ~60 substances in OF which can be easily adopted to changing requests.

- study 1: compare results OF screening results to routine urine drug testing with EIA.
  here: patients in opiate maintenance therapy.

- study 2: validation of 6-AM immunoassay for OF testing.
  same patient population



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.

Saliva: some data

-- production: up to 1.5 L/d, flow: 0.3-0.5 / 1.0-1.5 mL/min

-- Parotis(1) ~20%, Submandibularis(2) ~70%, Sublingualis(3) ~5%

-- **pH ~6.8**, after stimulation up to pH 7.8

-- "ingredients":

H_2O 99%
enzymes: **Amylase**
hormones: **Cortisol**
mucines
IgA and other Ig
electrolytes: Na^+, K^+, Cl^- , HCO_3^-
low flow rate: hypotonic
high flow rate: isotonic

How do drugs get into (mixed) saliva (oral fluid)?

-- oral contamination

-- from blood by **passive diffusion** across cell membranes

-- active secretion

-- filtration

factors influencing S/P-ratio:
-- **pKa** of substance (acidic-alkaline?)
-- lipid solubility
-- **protein binding**
-- molecular weight

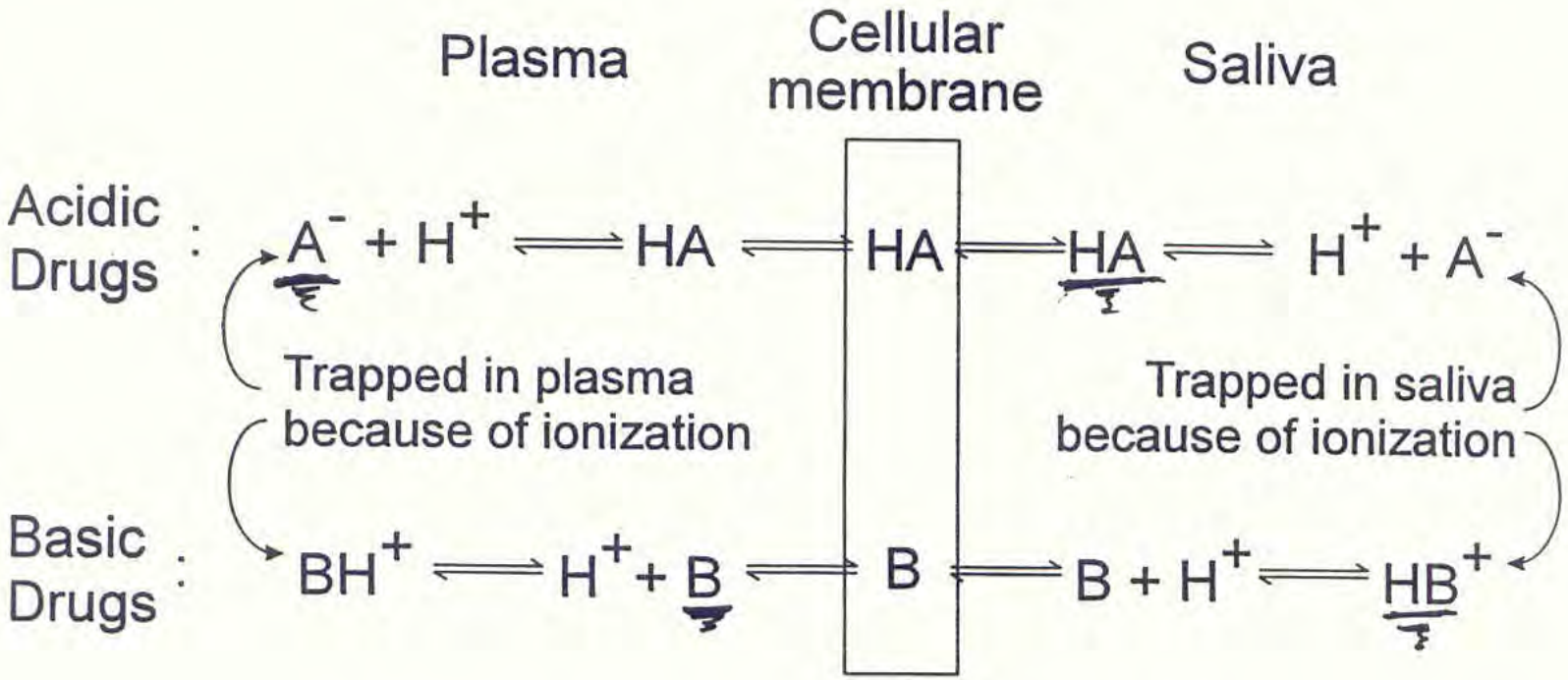
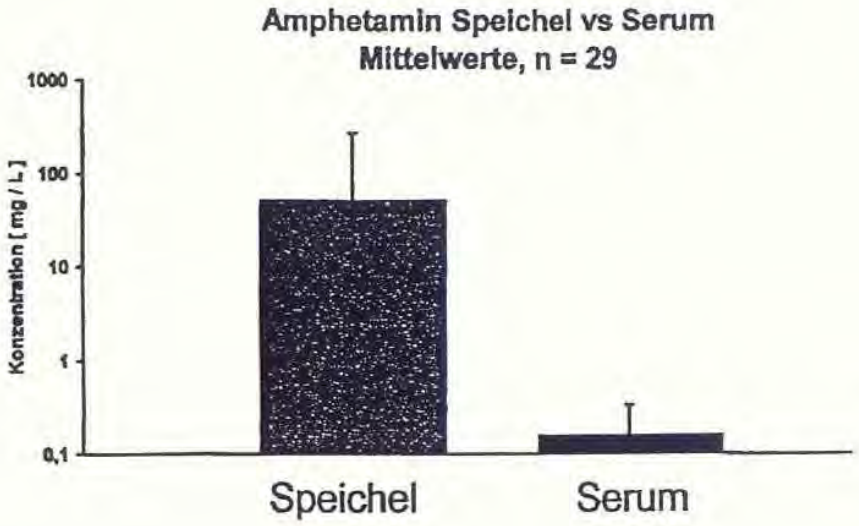
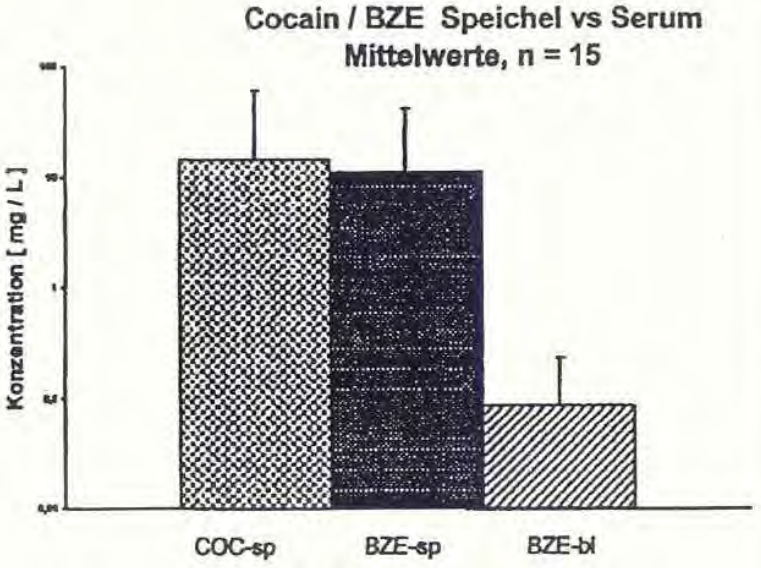
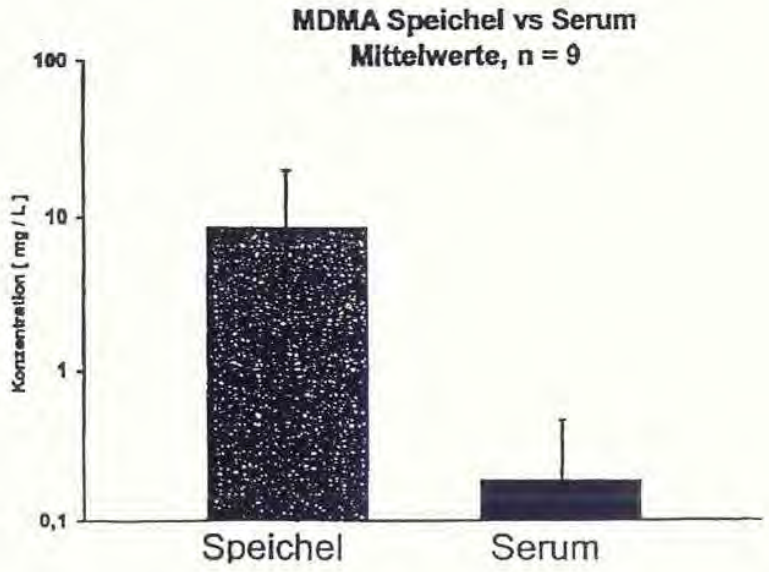
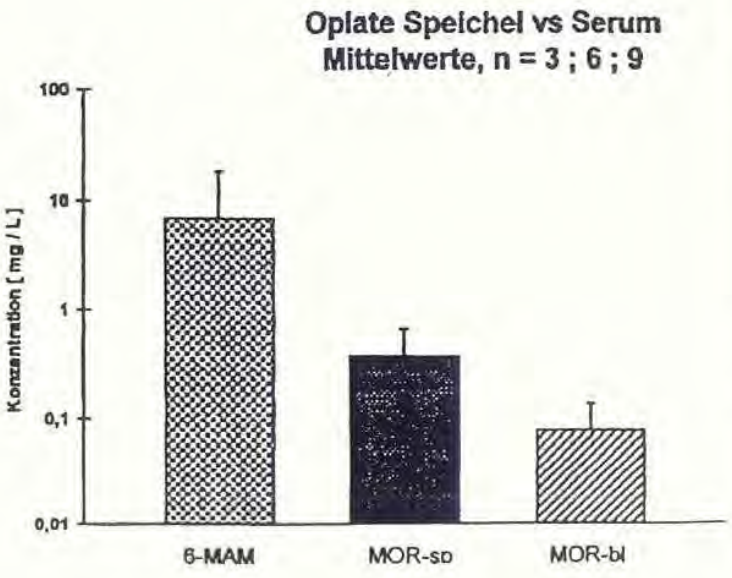


Fig. 1. Schematic diagram for transport of drugs into saliva or sweat.

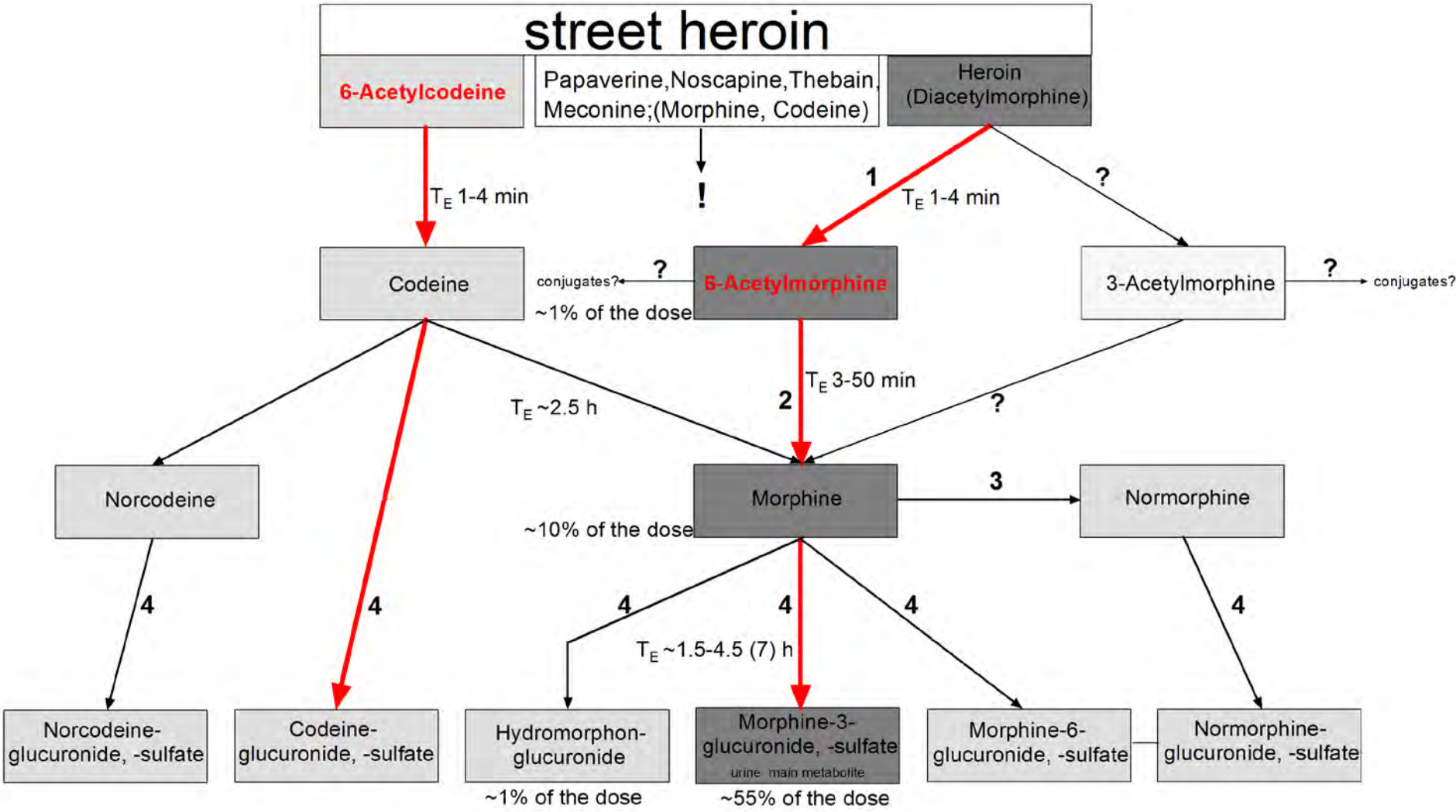








Kauert, Blutalkohol 37, 2000



(1) enzymatic (serum: Butyrylcholinesterase, liver: Carboxylesterases) and chemical.
(2) Acetylcholinesterases (erythrocytes) and Carboxylesterases (liver)
Demethylation of Morphine to Normorphine (3) is a minor pathway
Phase-II-Metabolismus (4) is polymorph (UGT2B7, UGT1A)

Oral Fluid is a Viable Alternative for Monitoring Drug Abuse: Detection of Drugs in Oral Fluid by Liquid Chromatography–Tandem Mass Spectrometry and Comparison to the Results from Urine Samples from Patients Treated with Methadone or Buprenorphine

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Abstract

Oral fluid is an alternative biological matrix that might have advantages over urine for drug analysis in treatment programs. A liquid chromatography–tandem mass spectrometry (LC–MS–MS) method has been used for screening 32 of the most commonly abused drugs and their metabolites in 0.5 mL preserved oral fluid, and the results were compared to results obtained from urine samples taken at the same time. In all, 164 pairs of oral fluid and urine were obtained from 45 patients stabilized on either methadone or buprenorphine. The total number of detections of drugs other than buprenorphine or methadone was 535 in oral fluid and 629 in urine. Morphine was found more often in urine (n = 66) than in oral fluid (n = 48), whereas the opposite was the case for 6-monoacetylmorphine (n = 20 in urine and n = 48 in oral fluid). Methadone showed the same detection frequency in urine and oral fluid (n = 75), whereas amphetamine (n = 45 in urine and n = 51 in oral fluid), methamphetamine (n = 39 in urine and n = 45 in oral fluid), and N-desmethyldiazepam (n = 37 in urine and n = 51 in oral fluid) were detected slightly more often in oral fluid. The other benzodiazepines, cannabis and cocaine were found more frequently in urine samples. If using a sensitive LC–MS–MS technique, oral fluid might be a good alternative to urine for detection of relatively recent use of drugs.

imens has accelerated over the last decade (1). An advantage with urine samples might be that drug ingestion can be detected for several days, and even weeks later, mainly because of detection of drug metabolites (2–4). However, urine may be difficult to collect; supervision intrudes on donors' privacy; the detection of drugs might be affected by, for example, the dilution of the urine due to fluid intake prior to urine sampling; and adulteration of the urine might render the analytical results worthless. Thus, there has been a growing interest in the use of oral fluid as an alternative to urine, and major technological advances have been made, particularly over the last 10 years (1). Collection of oral fluid is inoffensive, rapid, noninvasive, and easy, and the risk of adulteration is considered to be lower (5). Because of improved analytical techniques with increased sensitivity, a large number of drugs can be analyzed simultaneously in small sample volumes (6).

Oral fluid is a mixture of saliva, gingival crevicular fluid, cellular debris, and other components (5). Healthy adult subjects normally produce 500–1500 mL of oral fluid per day, at a rate of approximately 0.5 mL/min, but several physiological and pathological conditions can modify oral fluid production quantitatively and qualitatively (e.g., smell, taste stimulation, chewing, psychological and hormonal status, drugs, age, hered-

Table I. Cutoff Concentrations for Screening and Confirmation Analysis in Oral Fluid and Urine

Drug	Oral Fluid Analysis (ng/mL)	Urine Confirmation (ng/mL)	Urine Screening (ng/mL)
3-OH-Diazepam	3	150	
6-MAM	2	33	20
7-Aminoflunitrazepam	0.3	28	
7-Aminoclonazepam	1	29	
7-Aminonitrazepam	1	25	
Alprazolam	1	31	
α-OH-Alprazolam	NA*	32	
Amphetamine	1	135	300
Barbiturates			30
Benzodiazepines			200
Benzoylecgonine	14	58	
Bromazepam	16	32	
Buprenorphine	2		5
Buprenorphine-glucuronide	NA	5	
Norbuprenorphine-glucuronide	NA	12	
Cannabis			20
Carisoprodol†	52	1302	
Clonazepam	1	NA	
Codeine	3	60	
Cocaine	8	61	300
Diazepam	1	NA	
Fenazepam	2	3	
Flunitrazepam	1	NA	
Lorazepam	3	32	
LSD	0.3	0.03	0.50
MDA	36	1434	
MDEA	41	207	
MDMA	39	77	
Meprobamate†	44	1092	
Methadone	15	62	300
EDDP	NA	111	
Methamphetamine	3	149	
Morphine	6	29	
N-Desmethyldiazepam	1	135	
Nitrazepam	1	NA	
Opiates			300
Oxazepam	1	143	
THC-acid	0.3	10	
Zolpidem†	0.3	6	
Zopiclone†	2	4	

* Not analyzed.
† Only analyzed in urine if detected in oral fluid.

-- Intercept
-- LLE

Table II. Comparison of the Results from Oral Fluid and Urine Showing that the Results from the Sample Pairs Primarily Correspond

Drug	Positive OF* and Urine	Negative OF and Urine	Corresponding Results OF and Urine	Positive OF Only	Positive Urine Only
3-OH-Diazepam	6	117	123 (75%)	0	41
6-MAM	19	115	134 (82%)	29 !	1
7-Aminonitrazepam	9	149	158 (96%)	0	6
7-Aminoflunitrazepam	59	83	142 (87%)	3	19
7-Aminoclonazepam	26	122	148 (90%)	2	14
Alprazolam	9	153	162 (99%)	0	2
Amphetamine	45	113	158 (96%)	6 !	0
Benzoylecgonine	1	158	159 (97%)	0	5
Buprenorphine	67	–†		–	22 ?
Codeine	34	122	156 (95%)	4	4
Cocaine	0	161	161 (98%)	2	1
Methadone	75	89	164 (99%)	–	0
Methamphetamine	39	119	158 (96%)	6 !	0
Morphine	45	95	140 (85%)	3	21
N-Desmethyldiazepam	35	111	146 (89%)	16 !	2
Oxazepam	41	71	112 (68%)	9	43 !
THC/THCCOOH‡	81	64	145 (88%)	1	18
Zopiclone	4	106	110 (99%)	1	0

* Oral fluid.
† There were analytical problems with the oral fluid analysis.
‡ THC was analyzed in oral fluid, and THCCOOH was analyzed in urine.

screening for drugs: comparing OF-blood-urine

mixed Oral Fluid = saliva + gingival crevicular fluid + nasal secretions
+ mucosal transudates+ regurgitated gastric secretions

Oral Fluid	**Blood (Serum, Plasma)**	**Urine**
non invasive	invasive	supervision needed: privacy!
drug conc. low-high	drug conc. low	drug conc. low-very high
spl. vol. low	spl. vol. low	spl. vol. low-very high
adulteration difficult	no adulteration	adulteration possible
pH-change during collection process may influence Saliva/Plasma-ratio	-----	excretion influenced by urinary pH,drug concentration influenced by (intentional?!) drinking.
mostly parent drugs	parent drugs	mostly metabolites

screening for drugs: comparing OF-blood-urine

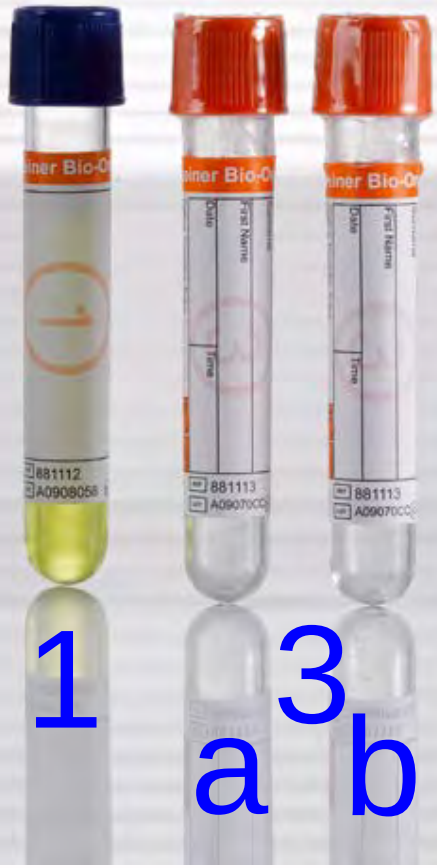
Oral Fluid	Blood (Serum, Plasma)	Urine
oral contamination from smoking,intranasal or peroral consumption	-----	-----
correlation with impairment could be possible	correlation with impairment possible	correlation with impairment impossible
screening methods, collection methods, collection devices not fully established and validated Adsorption!?	-----	screening methods, collection methods, collection devices established really standardized??
A+B sample?	-----	-----
collection device closed no contamination	closed device	urine beakers can be contaminated
Xerostomia	-----	"not able to..."

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

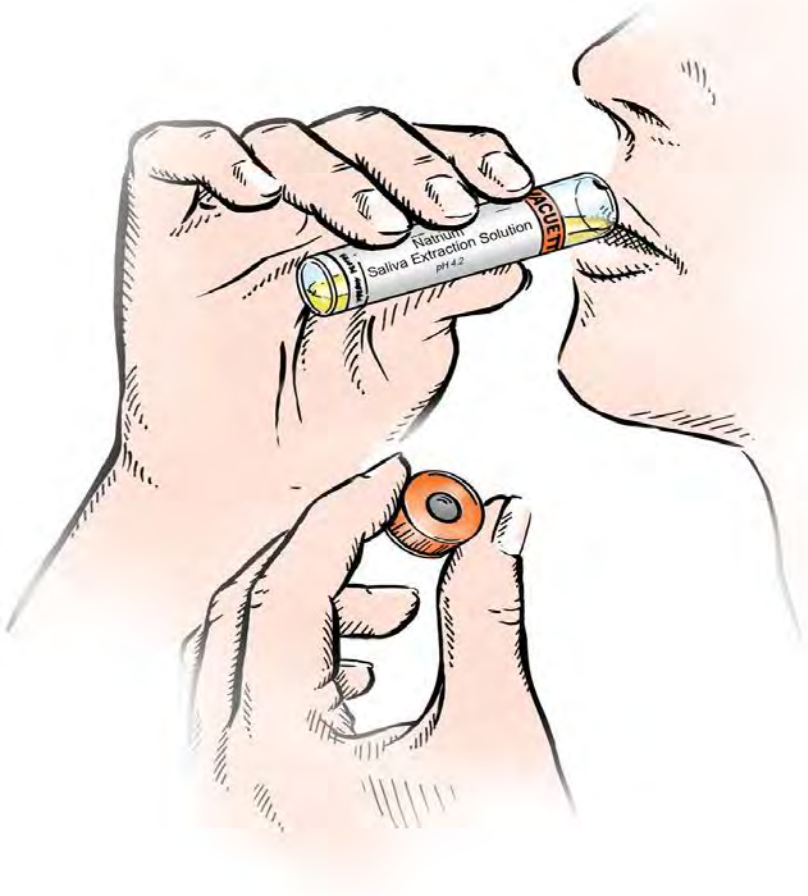
Saliva Collection Beaker
with integrated saliva transfer device



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

- **pH 4.5 citrate buffer**
- **Foodstuff color: OF conc. (OD)**

Step 2:

Spitting of the extracted oral fluid into the Saliva Collection Beaker

Step 3

Transfering of the extracted OF into the evacuated Saliva Collection Tubes

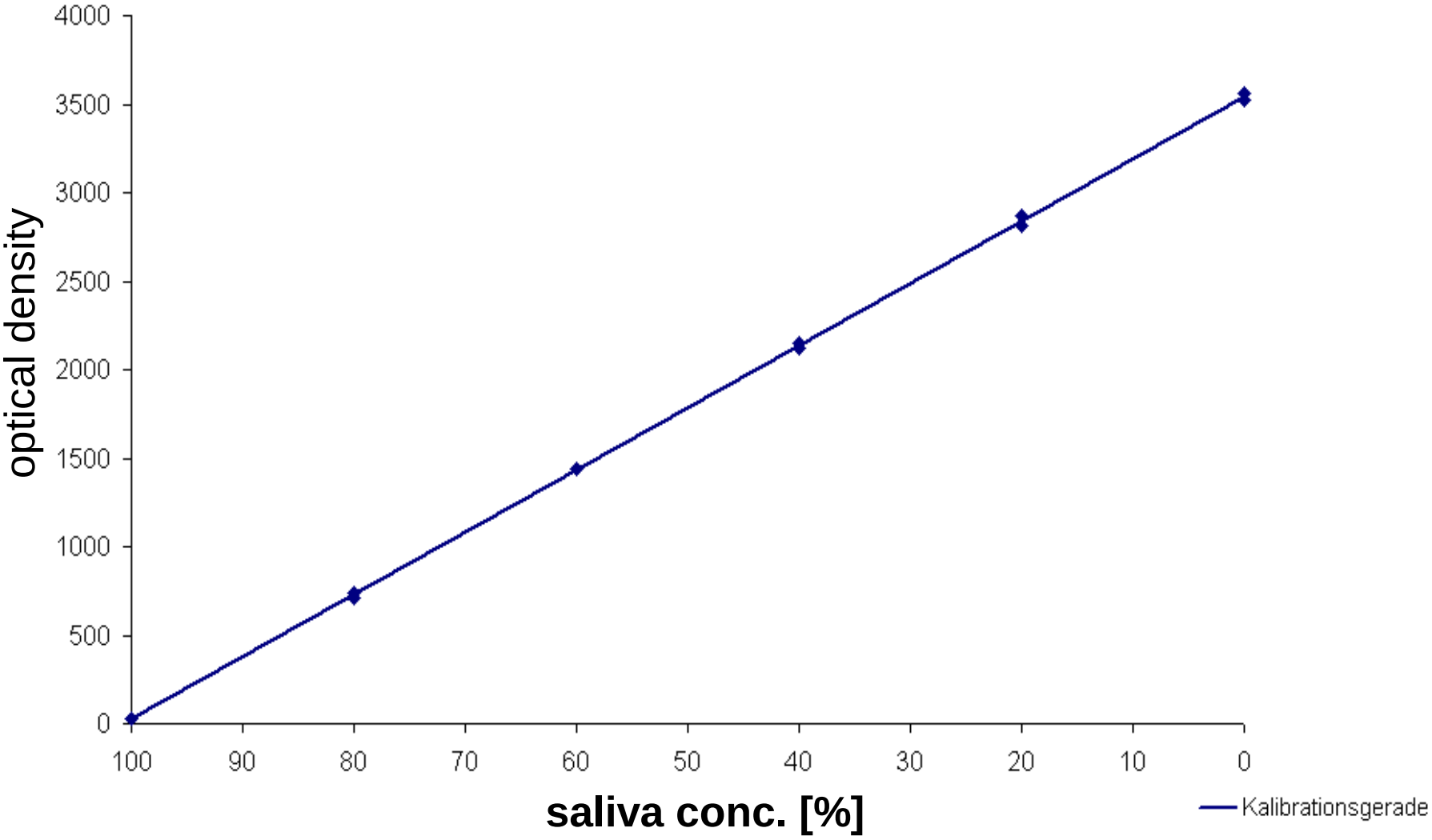
%OF is determined by photometry on Olympus AU680

always A + B sample!

Advantages:

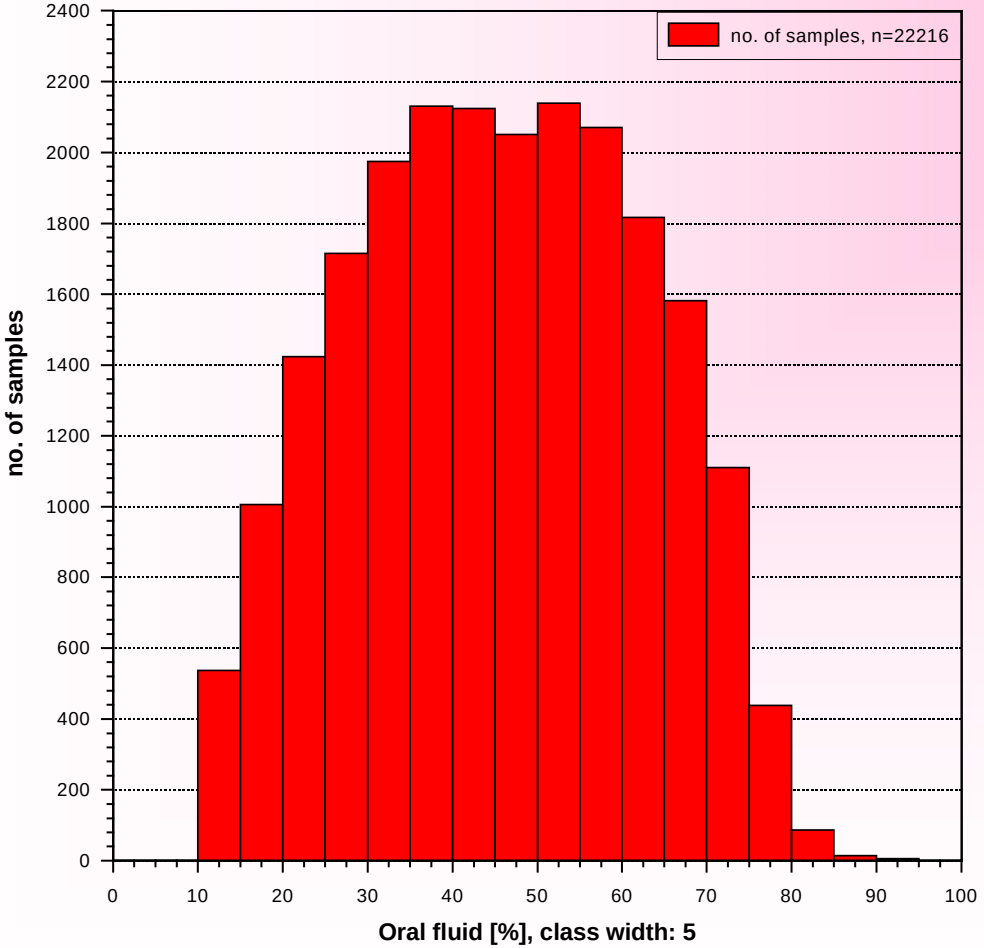
-- quick (Xerostomia!), standardized time
-- acidic pH during collection keeps pH difference to plasma
-- acidic pH: 6-AM, Cocaine, Zopiclone etc. are stable
-- aqueous matrix: less ion suppression, rapid SALLE possible
-- no bacterial growth

Saliva concentration [%]: calibration curve



Oral fluid collection with the GBO Saliva Collection System

Distribution of oral fluid concentrations



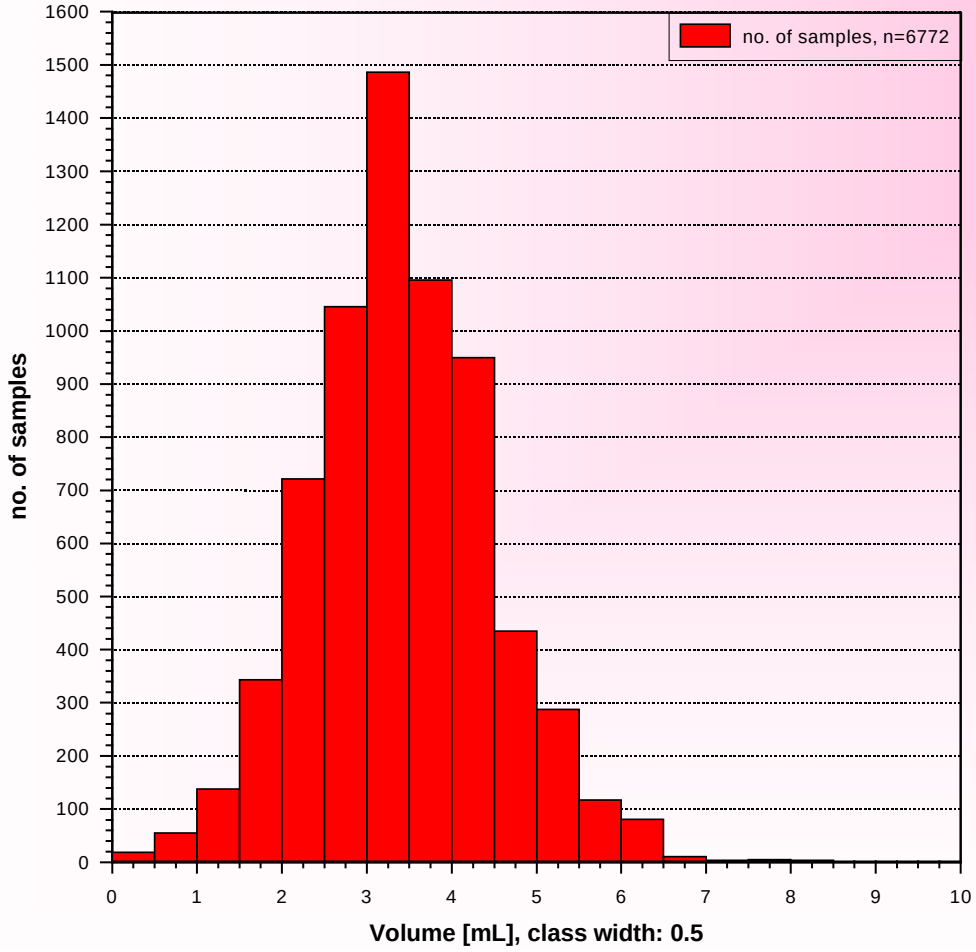
Statistics	Oral fluid [%]	n
mean	45.4	
median	45.4	
1% percentile	12.5	
5% percentile	18.1	
25% percentile	32.1	
75% percentile	58.7	
95% percentile	72.1	
99% percentile	77.9	
considered samples		22216
not considered	<10	252
not considered	nm*	52
all samples		22520

Samples from 4561 pats. (3313 male, 1248 female)
No gender information available for 219 pats.

- not measurable

Oral fluid collection with the GBO Saliva Collection System

Distribution of sample volume

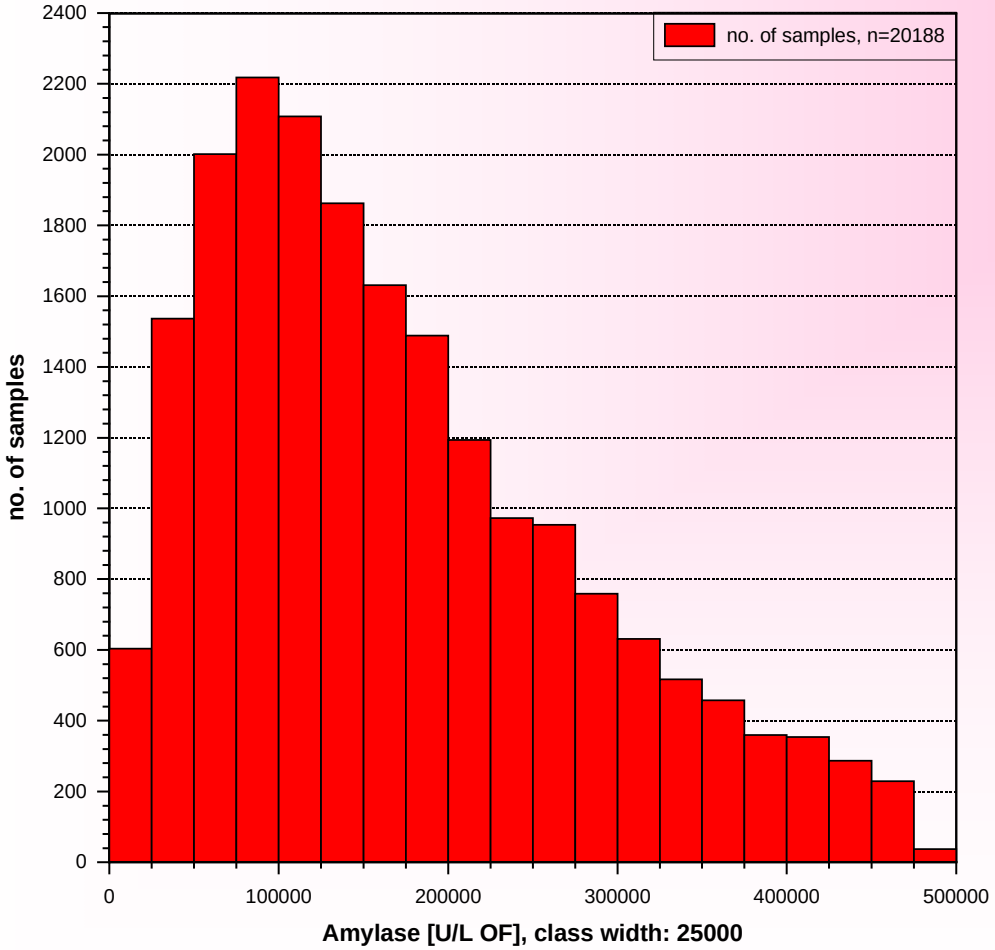


Statistics	Volume [mL]	n
mean	3.2	
median	3	
1% percentile	0.9	
5% percentile	1.5	
25% percentile	2.5	
75% percentile	4	
95% percentile	5	
99% percentile	6	
considered samples		6772
not considered	nm*	15748
all samples		22520

- not measurable

Oral fluid collection with the GBO Saliva Collection System

Distribution of amylase concentrations



Statistics	Amylase [U/L OF]	n
mean	170583	
median	146686	
1% percentile	15404	
5% percentile	33494	
25% percentile	85605	
75% percentile	236876	
95% percentile	392247	
99% percentile	456756	
considered samples		20188
not considered	>480000	1988
not considered	<10000	288
not considered	nm*	59
all samples		22520

- not measurable

Amylase activity + age: no change after 1st year

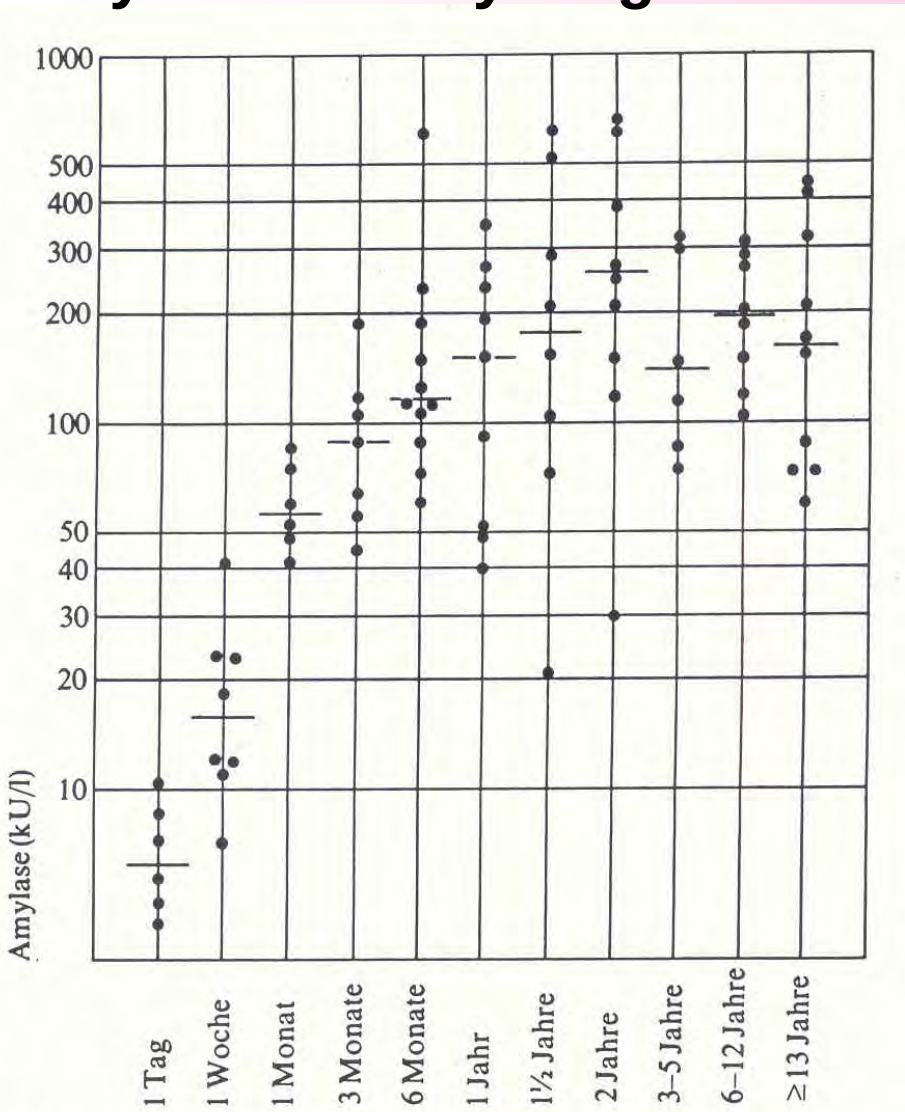


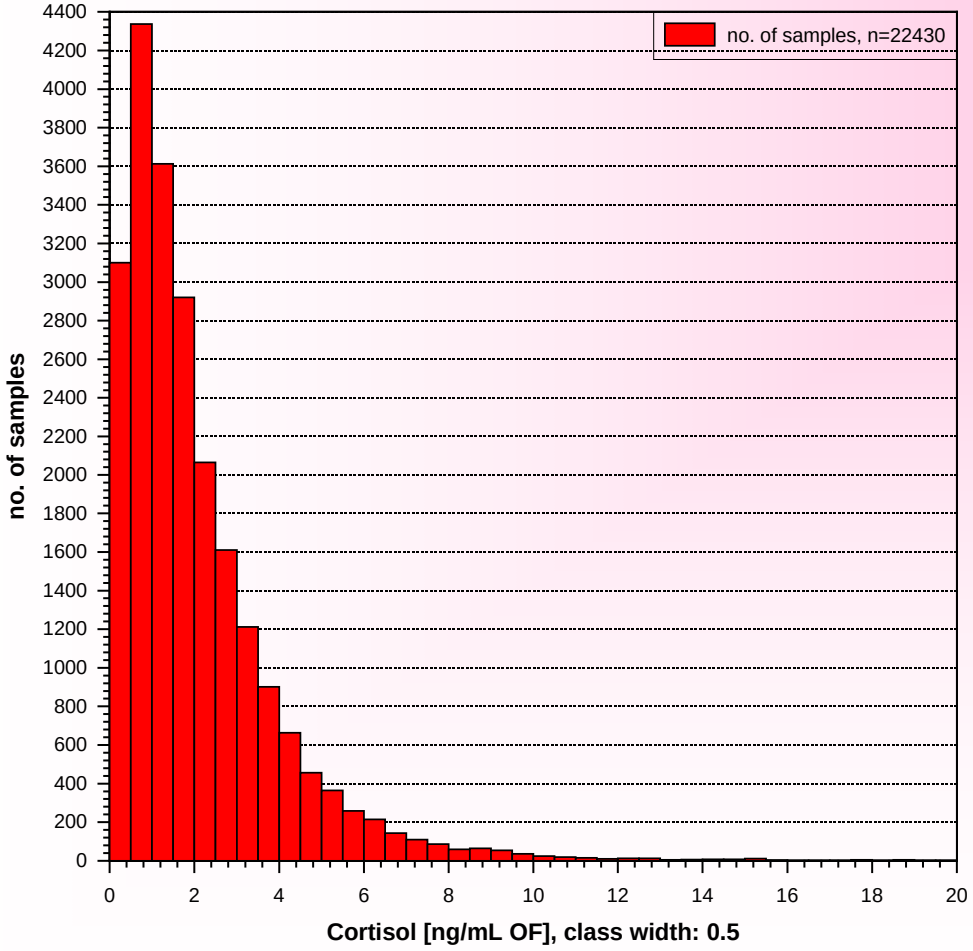
Abb.8. Amylaseaktivität in stimuliertem Gesamtspeichel in Abhängigkeit vom Alter[113].

Ciba Geigy, 1977

- There is no age related difference
- No sex related reference range
- Impact of medications?
- Impact of saliva flow?

Oral fluid collection with the GBO Saliva Collection System

Distribution of cortisol concentrations



Statistics	Cortisol [ng/mL OF]	n
mean	2.01	
median	1.5	
1% percentile	0.1	
5% percentile	0.2	
25% percentile	0.7	
75% percentile	2.7	
95% percentile	5.6	
99% percentile	9.2	
considered samples		22430
not considered	>20	46
not considered	<0.1	26
not considered	nm*	18
all samples		22520

- not measurable

Drug screening in Oral Fluid with UPLC-MS/MS: Analytes

Analytes in „**Module A“,** cutoff 0.1-1 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, Butylone, Mephedrone, Methylone, MDPV

- **Benzodiazepines**: Diazepam, Nordiazepam, Oxazepam, Midazolam, Flurazepam, Desalkyl-flurazepam, Temazepam, 7-Aminoclonazepam, Alprazolam, Flunitrazepam, 7-Aminoflunitrazepam, Bromazepam, Lorazepam, Phenazepam

- **Cocaine:** Cocaine, Benzoylecgonine, Methylecgonine, EME, AEME, Lidocaine

- **Opiates:** Morphine, Codeine, 6-Acetylmorphine, 6-Acetylcodeine, Norcodeine, Dihydrocodeine

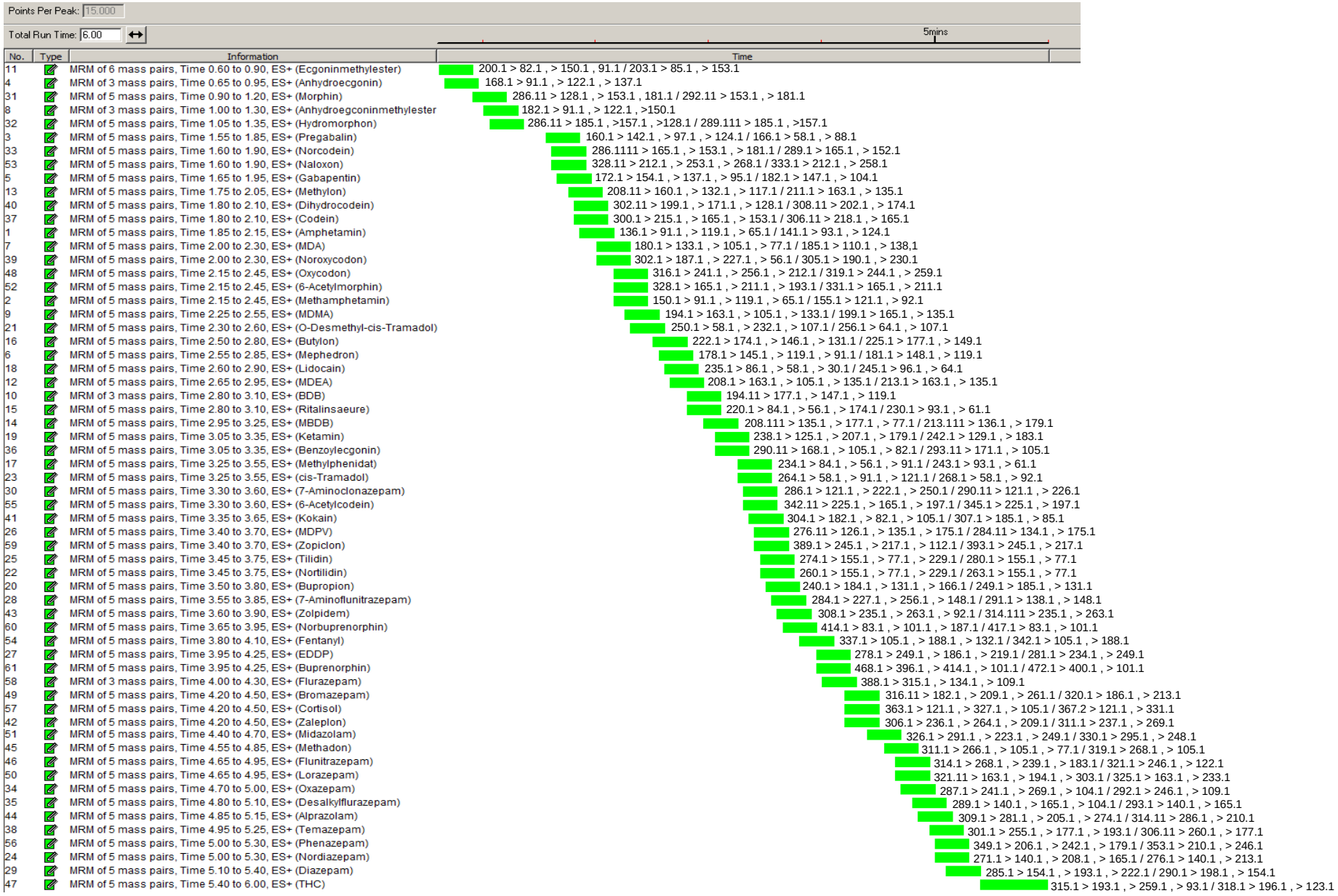
- **Opioids:** Naloxone, Tilidine, Tramadol, O-Desmethyltramadol, Oxycodone, Noroxycodone, Fentanyl, Nortilidine, Hydromorphone

- **Cannabinoids:** THC

- **Others:** Zolpidem, Zopiclone**,** Zaleplone, Ketamine, Methylphenidate, Ritalinic acid, Pregabalin, Gabapentin, Bupropion

actual: N = 61 (3 transitions) **+ 57 deuterated IS** (2 transitions)

Drug screening with UPLC-MS/MS: chromatographic separation



Method Description:

Saliva and Amylase (BeckmanCoulter) concentration (authenticity markers)
is measured on an Olympus AU680

UPLC-MS/MS method:

system:	Waters UPLC-MS/MS Xevo TQ-S
injection volume:	5 µL
Mopha A:	H_2O + 0.1 % formic acid + ammonium formate 20 mmolar
Mopha B:	MetOH + 0.1 % formic acid
flow:	0.55 mL/min
column:	Waters BEH Phenyl 1.7 µm, 2.1 x 150 mm
column T.:	60°C
run time:	6.0 min
analytes:	61 + 57 deuterated standards

Sample Preparation:

- 100 µL saliva spl.
+ 10 µL deuterated standard (5 ng/mL / 0.5 ng/mL spl. / 50 pg)
- vortex
+ 50 µL sulpho salicylic acid (20 %)
+ 600 µL ACN
+ 100 µL ammonium formate (10 molar)
- vortex
- centrifuge (1 min ; 13400 rpm / Eppendorf Mini Spin), phase separation!
- pipette 550 µL of the supernatant (organic phase) into a glas insert
+ 10 µL ethylenglycol
- evaporate (45°C ; nitrogen-stream 2 bar / 30 psi ; ~ 20 min)
redissolve in 80 µL H_2O + 10 µL MetOH

Drug screening in oral fluid with LC-MS/MS: Chromatographic conditions

Column:	ACQUITY UPLC® BEH Phenyl 1.7µm, 2.1mm x 150mm
Column heater:	60°C
Mobile Phase A:	ammonium formate 5 mmol/L + 0.1% formic acid (pH 3)
Mobile Phase B:	methanol + 0.1% formic acid
Run time:	6.0 min
Flow:	0.55 mL/min

Gradient:

0.00 min, 85% mobile Phase A + 15% mobile Phase B
3.00 min, 45% mobile Phase A + 55% mobile Phase B (curve)
4.00 min, 45% mobile Phase A + 55% mobile Phase B
5.00 min, 0% mobile Phase A + 100% mobile Phase B
6.00 min, 0% mobile Phase A + 100% mobile Phase B

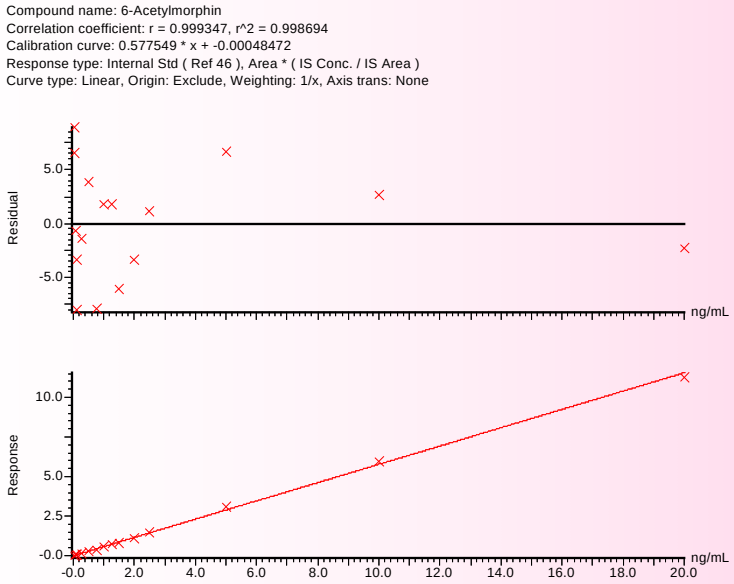
Followed by "blank injection" method:

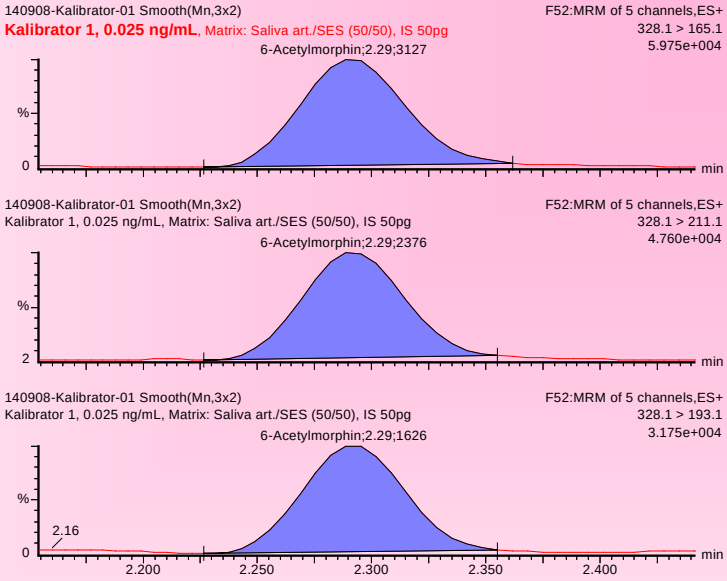
0.00 min, 85% mobile Phase A + 15% mobile Phase B
0.50 min, 85% mobile Phase A + 15% mobile Phase B

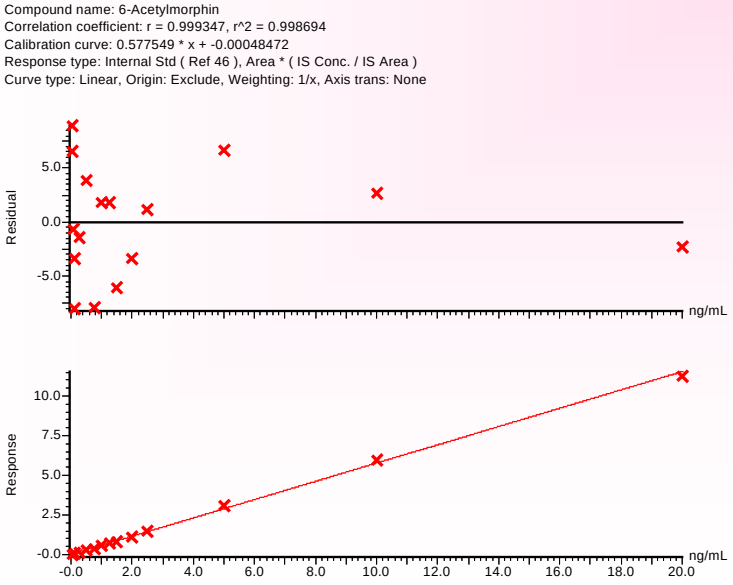
Detector settings Waters Xevo TQ-S

- ESI positive ionization mode
- capillary voltage: 0.2 kV
- ion source T.: 150°C
- desolvation gas (N_2): 650°C, 1000 L/h
- cone gas (N_2): 150 L/h
- collision gas (Ar): 0.17 mL/min

6-AM with UPLC-MS/MS: calibration curve







UPLC-MS/MS

16-point calibration:

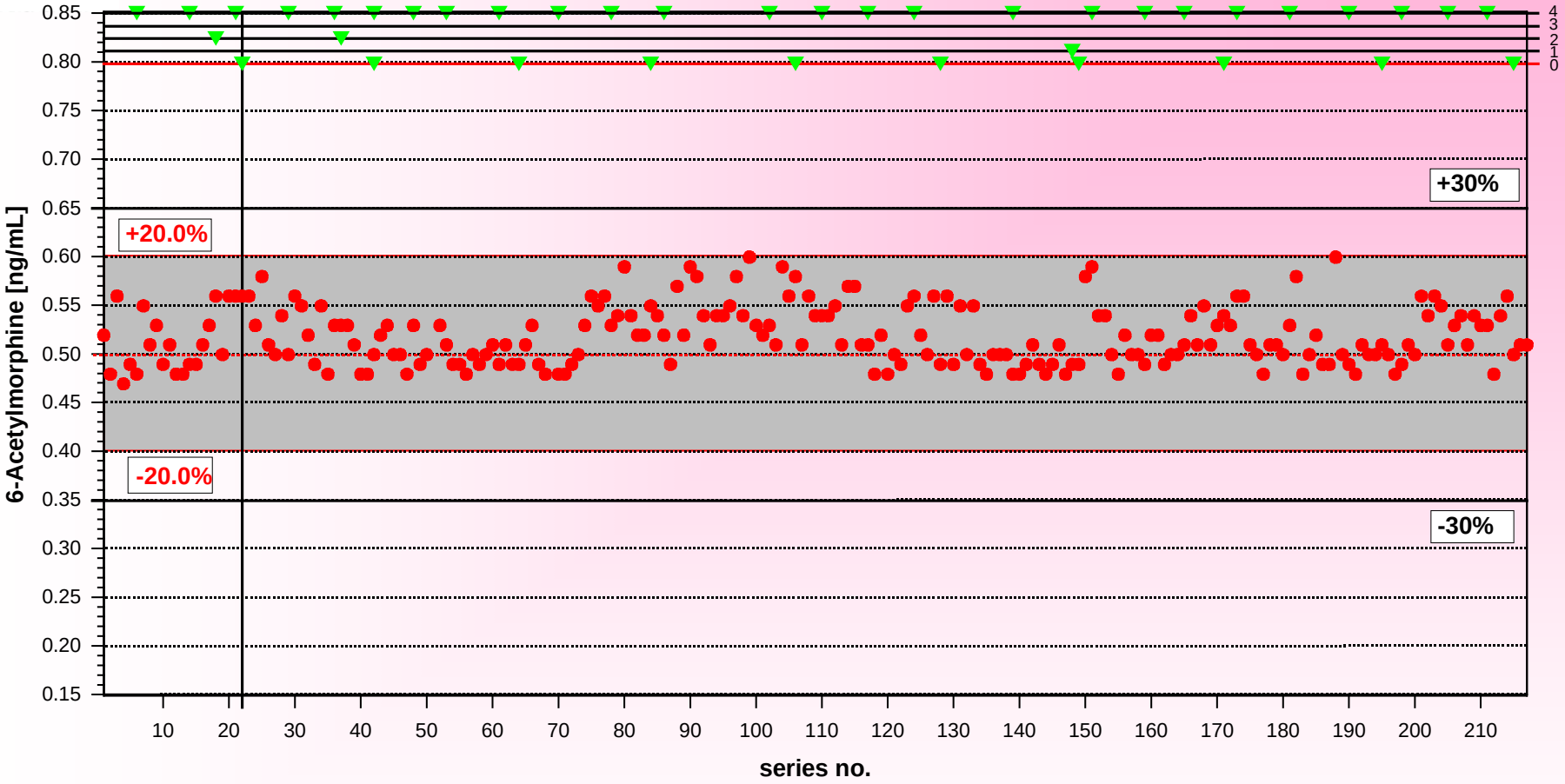
0.025, 0.05, 0.075, 0.1, 0.125,0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 2.0, 2.5, 5.0, 10.0, 20.0

QC:

A lyophilized pool saliva (OF/SES = 60%) spiked at 0.6 ng/mL (= 1 ng/mL neat OF)

MVZ Labor Dessau GmbH

Screening for drugs of abuse in oral fluid with UPLC-MS/MS
6-Acetylmorphine in 62% pool OF / SES
Control: QC oral fluid screening, Lot: 21112012, exp. date: n.a.



- 6-Acetylmorphine, oral fluid-ctrl.
  target value (GTFCh) ±30%: 0.35-**0.50**-0.65 [ng/mL]
  allowed relative deviation (according to RiliBÄK): **20.0%**

0.40-0.50-0.60

- 0 = end of control cycle
- 1 = new IS and new calibration
- 2 = new calibration
- 3 = new IS
- 4 = QC reconstituted

1st Study: is OF of equal value?

Drug abuse testing of patients in substitution therapy: UPLC-MS/MS screening in OF vs. urine testing with EIA

-- three month observation period

-- urine cutoffs: Amphs 500 ng/mL, Benzos (enzym. hydrolysis) 100 ng/mL, Coca 50 ng/mL, Opi 100 ng/mL, EDDP 100 ng/mL, Bupre 2 ng/mL, THC-COOH 25 ng/mL.

-- saliva cutoffs: 0.1-1 ng/mL (neat OF)

-- Patients from:

1. an outpatient clinic **(OPC)** where the drug testing was stepwise moved from urine to SA.
   - **194 patients** (26 Bupre, 67 Metha, 101 Pola), **902 SA** samples.
   - **182 patients** (25 Bupre, 66 Metha, 91 Pola), **1119 urine** samples.

2. other outpatient clinics **(ALL)** with more random selection between the two matrices.
   - **612 patients** from 23 clinics (116 Bupre, 265 Metha, 231 Pola), **1072 SA** samples.
   - **1463 patients** from 40 clinics (285 Bupre, 673 Metha, 505 Pola), **9008 urine** samples.

Drug abuse testing of patients in substitution therapy: UPLC-MS/MS screening in saliva vs. urine testing with EIA

	OPC	OPC	OPC	ALL	ALL	ALL
	saliva % pos. spls.	urine % pos. spls.	urine no. of spls.	saliva % pos. spls.	urine % pos. spls.	urine no. of spls.
Amphetamines	9.3	3.3	1082	10.3	4.1	7396
Benzodiazepines	11.0	14.4	958	25.7	22.4	6891
Cocaine	5.2	3.9	1075	9.8	7.2	8295
Opiates	13.5	13.5	968	17.6	21.7	6977
Methadone saliva EDDP urine	86.6	85.2	953	85.4	88.0	8938
THC	26.9	-	-	30.5	31.3	598
Opioids	1.2	-	-	2.1	-	-
Others	0.8	-	-	1.4	-	-
Buprenorphine	12.3	-	-	16.9	73.1	640
	n = 902			**n = 1072**		

Methadone/EDDP was positive in both matrices where expected.
However, Buprenorphine was negative in 8 OF samples from 2 OPC patients in low dose therapy (0.4 and 1.0 mg/d).
Cutoff 0.1 ng/mL?

Detailed results OF II

	OPC	OPC	ALL	ALL
	saliva % pos. spls.	saliva % from pos.	saliva % pos. spls.	saliva % from pos.
Opiates	13.5	100	17.6	100
Morphine	13.3	98.4	16.9	95.8
6-Acetylmorphine	10.3	**76.2**	13.4	**76.2**
Codeine	8.2	60.7	12.8	72.5
6-Acetylcodeine	3.7	**27.0**	4.8	27.0
Norcodeine	2.8	20.5	4.1	23.3
Dihydrocodeine	0.1	0.8	0.1	0.5
Methadone saliva **EDDP** urine	86.6	100	85.4	100
THC	26.9	100	30.5	100
Opioids	1.2	100	2.1	100
Fentanyl	-	-	0.5	21.7
Tramadol	0.8	63.6	0.8	34.8
Tilidine	0.2	18.2	0.4	17.4
Naloxone	0.2	18.2	0.4	17.4
Oxycodone	-	-	0.2	8.7
Others	0.8	100	1.4	100
Ketamine	0.7	85.7	0.6	40.0
Zopiclone	0.1	14.3	0.4	26.7
Zolpidem	-	-	0.3	20.0
Methylphenidate	-	-	0.2	13.3
Buprenorphine	12.3	100	16.9	100

Cutoff considerations: a retrospective study
All routine OF samples, 3 month

Samples: 5355

from pats. in maintenance therapy:	4954 spls. = 92.5% of all spls.
from Methadone/Polamidone™ pats.:	3671 spls. = 68.5% of all spls.
from Buprenorphine pats.:	1283 spls. = 24.0% of all spls.

Patients: 2050

male: 1455 (71.0%), female: 595 (29.0%)

in maintenance therapy:	1877 pats. = 91.6% of all pats.
male:	1347 pats. = 65.7% of all pats.
female:	530 pats. = 25.9% of all pats.
Methadone/Polamidone™ pats.:	1315 pats. = 64.1% of all pats.

male: 924 (63.5%), female: 391 (36.5%)

Buprenorphine pats.:	562 pats. = 27.5% of all pats.

male: 423 (75.3%), female: 139 (24.7%)

Benzodiazepines :

CO 1 ng/mL: 731 pos. samples = 13.7% **CO 10 ng/mL: 415 pos. samples = 7.7%**

a sample was defined positive when at least one analyte was >= CO

Positive samples rate reduced by 43.2%

No. of spls.	Analytes >= CO 1 ng/mL	No. of spls.	Analytes >= CO 10 ng/mL	reduced by
663	Nordiazepam	336	Nordiazepam	49.3%
536	Diazepam	239	Diazepam	55.4%
343	Oxazepam	51	Oxazepam	85.1%
182	Temazepam	17	Temazepam	90.7%
38	Lorazepam	18	Lorazepam	52.6%
32	7-Aminoclonazepam	17	7-Aminoclonazepam	46.9%
30	Bromazepam	24	Bromazepam	20.0%
12	Alprazolam	5	Alprazolam	58.3%
5	7-Aminoflunitrazepam	0	7-Aminoflunitrazepam	100.0%
1	Midazolam	0	Midazolam	100.0%

Most of the positive samples are related to Diazepam ingestion. Because of its elimination half-life (~100 h) and its better OF/plasma-ratio when compared with the other Diazepam metabolites, Nordiazepam determines the positive sample rate. Nordiazepam is the target analyte in OF to detect Diazepam consumption. The Lorazepam cutoff should perhaps be lowered. For the other Benzodiazepines more data are needed.

Opiates :

CO 1 ng/mL: **610 pos. samples = 11.4%** **CO 10 ng/mL:** **397 pos. samples = 7.4%**

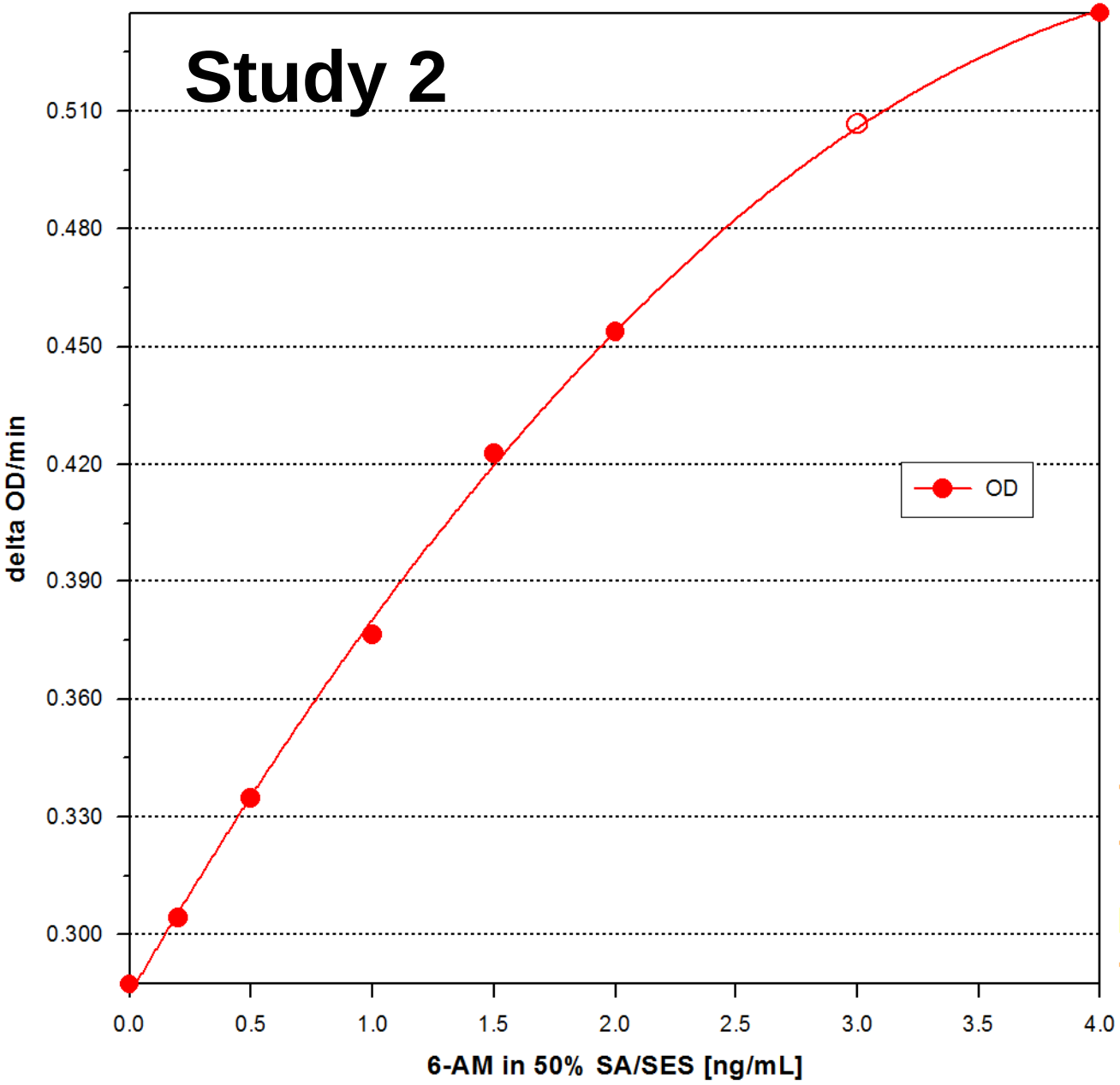
a sample was defined positive when at least one analyte was >= CO

Positive samples rate reduced by 34.9%

No. of spls.	Analytes >= CO 1 ng/mL	No. of spls.	Analytes >= CO 10 ng/mL	reduced by
597	Morphine	376	Morphine	37.0%
494	6-Acetylmorphine	237	6-Acetylmorphine	52.0%
396	Codeine	217	Codeine	45.2%
173	6-Acetylcodeine	100	6-Acetylcodeine	42.2%
129	Norcodeine	10	Norcodeine	92.2%
11	Dihydrocodeine	6	Dihydrocodeine	45.2%

81.0%	of all Opiate positive samples contained 6-Acetylmorphine thus proving Heroin abuse.	60.0%	of all Opiate positive samples contained 6-Acetylmorphine thus proving Heroin abuse.
34.7%	of all 6-Acetylmorphine positive samples contained 6-Acetylcodeine thus proving "Street Heroin" abuse.	42.2%	of all 6-Acetylmorphine positive samples contained 6-Acetylcodeine thus proving "Street Heroin" abuse.

New 6-AM OF CEDIA: calibration curve Olympus AU 680



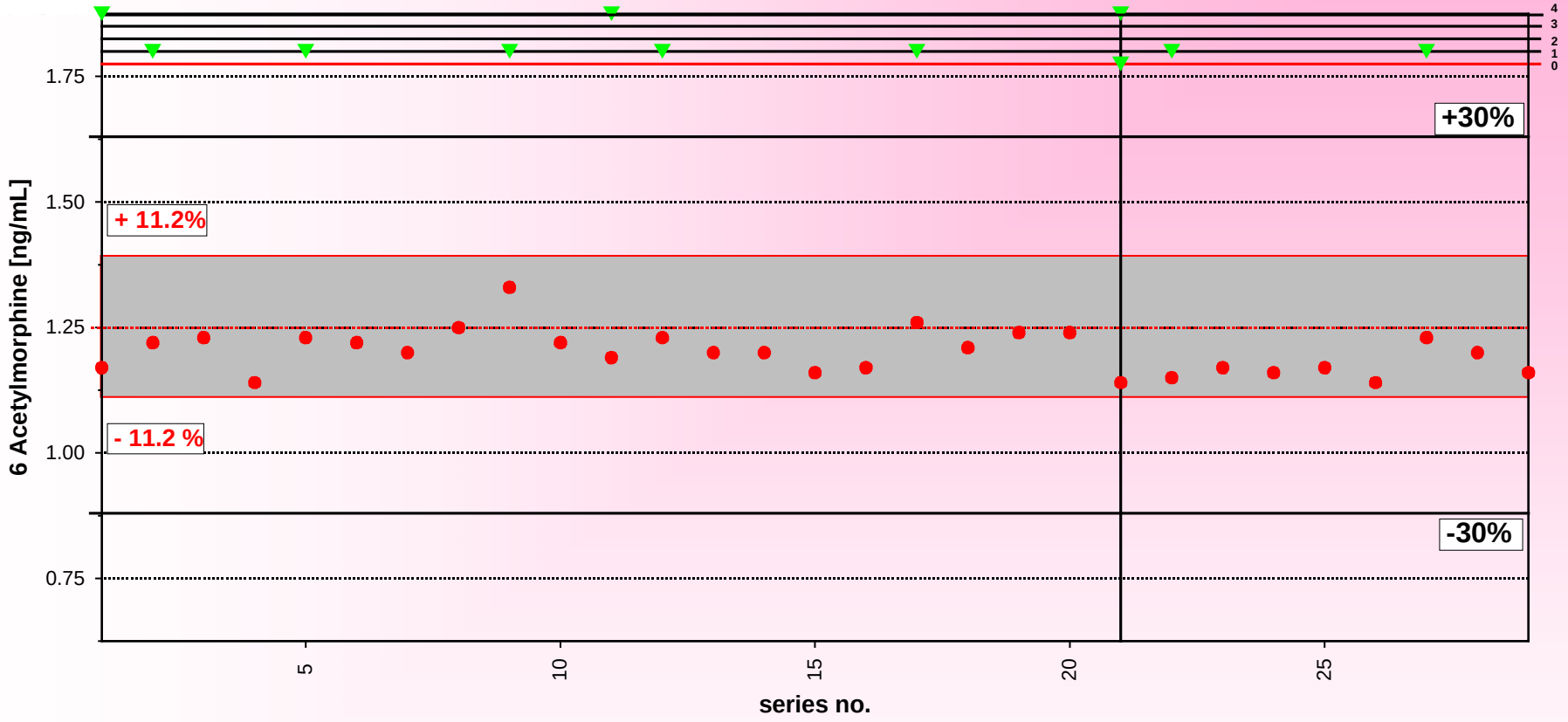
OD

Polynom - Regression: (N = 8)
Goodness of Fit:
Chi^2 = 0, p = 0
y = a + bx + ...
0E+000 <= x <= 4
a = 0.2847 ±0.6883
b = 0.1065 ±0.9403
c = -0.0109 ±0.2331
Geschätzt mit 1/n:
Varianz der Residuen = 4.2386E-006
Stdabw. der Residuen = 0.0021
Geschätzt mit 1/(n-3):
Varianz der Residuen = 6.7817E-006
Stdabw. der Residuen = 0.0026
Korrelationskoeffizient = 0.9997
df = 5
p = 0.00004
Eta^2 = 0.9994
$Eta^2_{adj.}$ = 0.9992

ThermoFisher (Microgenics):
-- no calibrators at that time
lowest cal. at 0.25 ng/mL
because CO 1 ng/mL neat OF
-- no control samples

MVZ Labor Dessau GmbH

Screening for drugs of abuse in oral fluid on Olympus AU 680
6-Acetylmorphine in 50% artef. OF / SES
Control: QC oral fluid screening **high control**, Lot: 28072014, exp. date: n.a.



6-Acetylmorphine, oral fluid low control
target value(gravim.) ±30% : 0.88 - **1.25** - 1.63 [ng/mL]
allowed relative deviation(according to RiliBÄK) : 11.2%

1.11 - 1.25 - 1.39

6-Acetylmorphine, oral fluid low control
0 = end of control cycle
1 = new calibration
2 = new calibration (QC out of range)
3 = new calibration (new reagent)
4 = new QC sample reconstituted

2nd study: 6-AM OF CEDIA validation study
A Opiates negatives

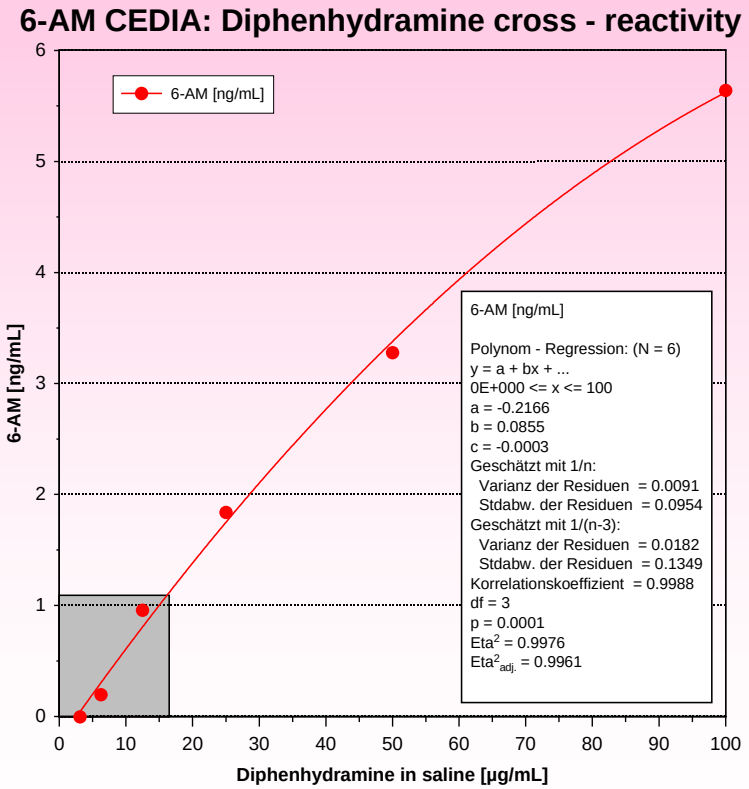
100 Opiates negative samples, 100 patients,
Pats. in Opiates substitution therapy
Routine samples from 1 week

Negative with LC/MS-MS:
LC-MS/MS LoQ <0.25 ng/mL neat OF

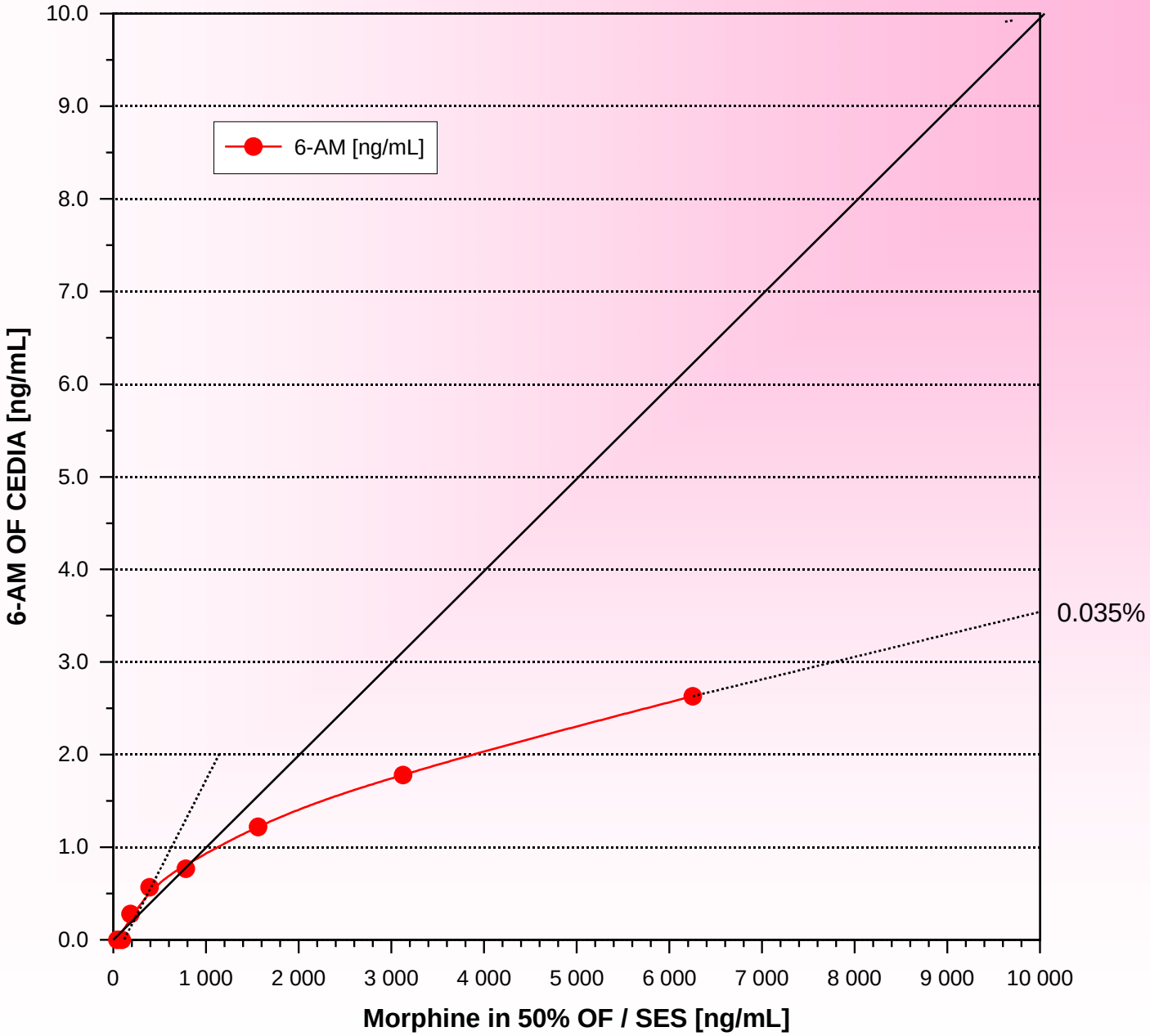
1 sample 6-AM CEDIA positive = 1.1 ng/mL
(1.7 ng/mL in neat OF (62%))

Contained 16.5 µg/mL Diphenhydramine
(26.8 µg/mL in neat OF)

99 spls. negative in 6-AM OF CEDIA
(0 ng/mL)



6-AM OF CEDIA: Morphine cross-reactivity



2nd study: 6-AM OF CEDIA validation study
B Opiates positives

4039 samples, ~900 patients,
~90% in Opiates substitution therapy
Routine samples from 6 consecutive weeks

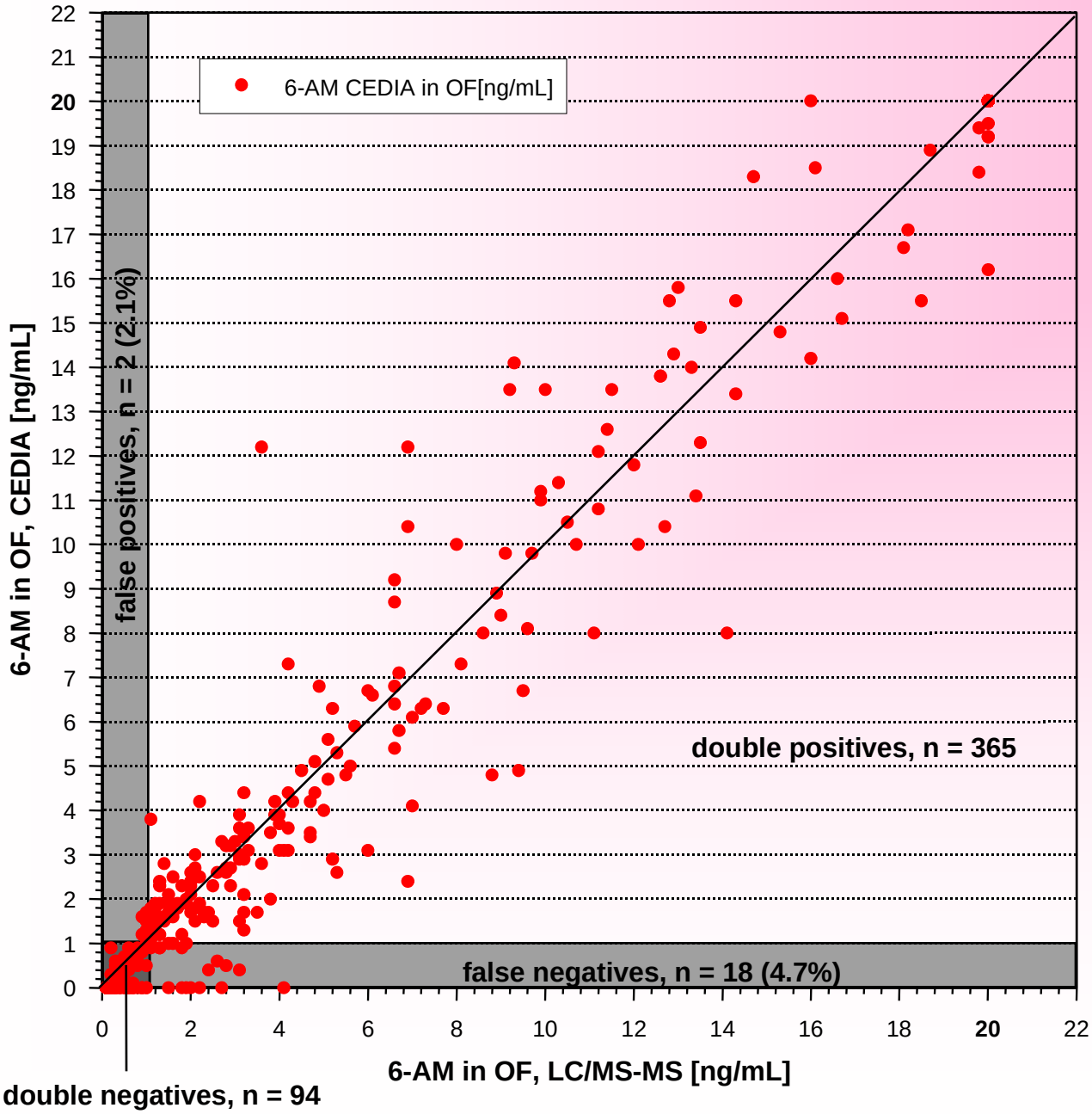
LC-MS/MS cutoff 1 ng/mL neat OF

Opiates positive LC/MS-MS
n = 481 = 11.9%

Opiates positive,
6-AM positive LC/MS-MS
n = 385 = **80.0%** of Opiates positives
9.5% of all samples

Opiates positive,
6-AM negative LC/MS-MS
n = 96 = 20.0% of Opiates positives

Agreement of 6-AM CEDIA and LC-MS/MS



Study

4039 samples, ~900 patients
6 weeks
Opiates positive LC/MS-MS:
n = 481 = 11.9%

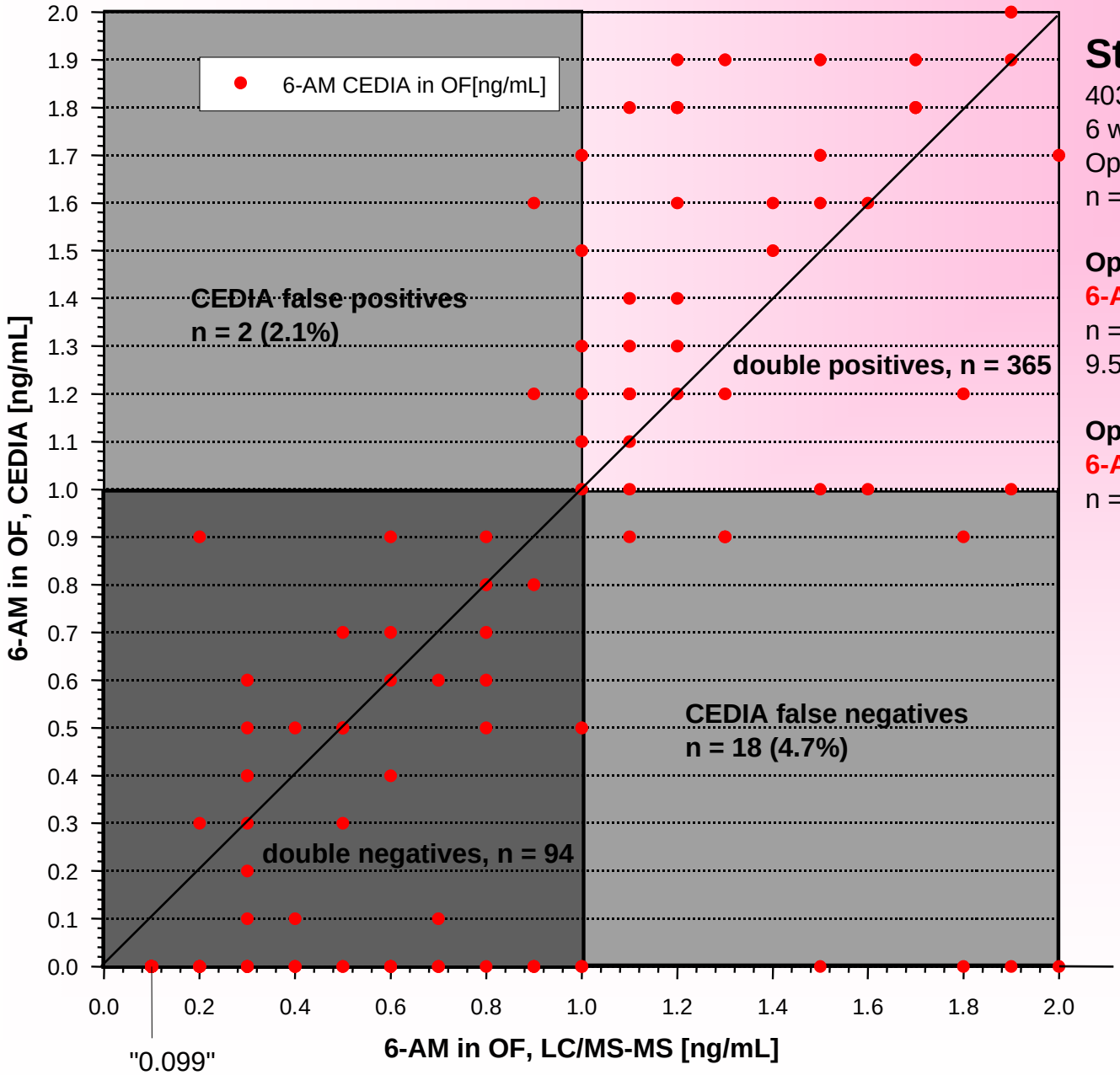
Opiates positive,
6-AM positive LC/MS-MS
n = 385 = 80.0% of Opiates positive
9.5% of all samples

Opiates positive,
6-AM negative LC/MS-MS
n = 96 = 20.0% of Opiates positive

6-AM CEDIA in OF[ng/mL]

Lineare Regression: (N = 385)
y = a + bx
0E+000 <= x <= 21
a = -0,1231
b = 1,0038
Geschätzt mit 1/n:
Varianz der Residuen = 1,5459
Stdabw. der Residuen = 1,2434
Geschätzt mit 1/(n-2):
Varianz der Residuen = 1,554
Stdabw. der Residuen = 1,2466
Korrelationskoeffizient = 0.9883
df = 383
p <= 0.00001
Eta^2 = 0.9768
$Eta^2_{adj.}$ = 0.9767

Agreement of 6-AM CEDIA and LC-MS/MS, low conc. range



Study

4039 samples, ~900 patients
6 weeks
Opiates positive LC/MS-MS:
n = 481 = 11.9%

Opiates positive,
6-AM positive LC/MS-MS
n = 385 = 80.0% of Opiates positive
9.5% of all samples

Opiates positive,
6-AM negative LC/MS-MS
n = 96 = 20.0% of Opiates positive

Conclusions for 6-AM

-- The new 6-AM CEDIA for oral fluid is a rapid and sensitive assay to spot recent Heroin abuse in Opiates addicts.

-- There were no false positives due to cross-reactivity with Morphine or Dihydrocodeine. Cave! Oral contamination..... One false positive with Diphenhydramine.

-- 11.9% of the samples from the investigated patient population were Opiates positive (cutoff 1 ng/mL) with LC/MS-MS. From these ~76% (80%) were positive for 6-AM with CEDIA (LC-MS/MS) at 1 ng/mL cutoff.

-- Specificity: only 2 samples from 4039 (481 Opiates positives) were false positive. These samples had 6-AM below the cutoff.
-- Sensitivity: 18 samples from 385 true 6-AM positives were false negative (4.7%). Mostly samples with low saliva concentration (<20%).

Conclusions for OF vs urine testing

-- Amphetamines and Cocaine/Benzoylecgonine better in OF.

-- 6-AM higher positive rate in OF than urine. Opiates positive rate comparable.

-- Benzodiazepines with shorter detection time window. However, urine detection time sometimes too long.

-- Positive rate for Methadone and Buprenorphine is comparable. Cutoff 0.1 ng/mL for Buprenorphine, Norbuprenorphine and EDDP.

-- THC: only oral contamination in OF. Short detection time sometimes helpful (~12 hours) because urine samples can be positive for >7 days.

Thank you for your attention!

michael.boettcher@laborpraxis-dessau.de

"**Saliva** is not one of the popular body fluids. It lacks the drama of blood, the sincerity of sweat and the emotional appeal of tears."

ID Mandel
J. Oral Pathological Medicine, 1990

Trias: speed, price, sensitivity
⟶ Immunoassays

-- excluding additional drug consumption from patients in substitution therapy

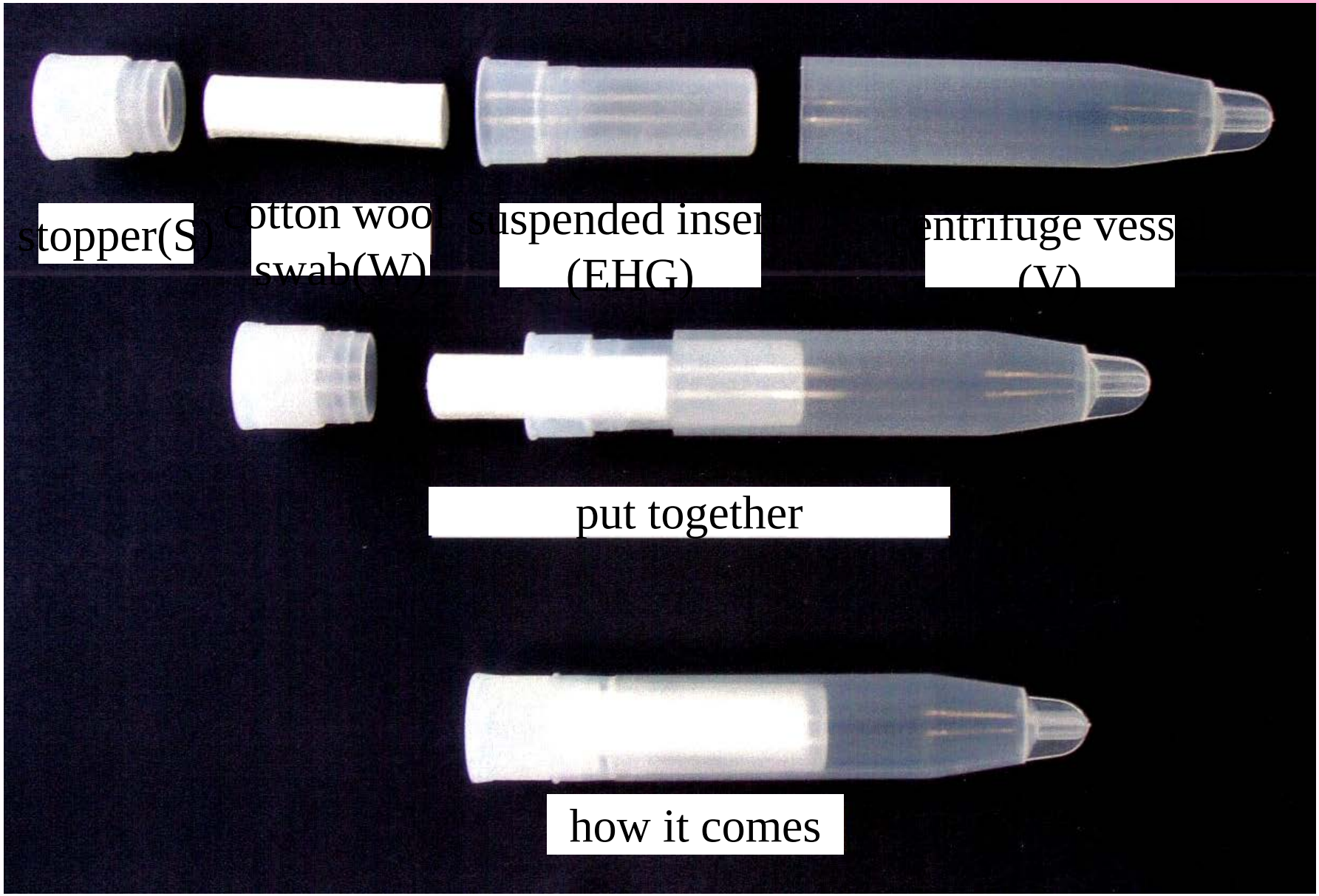
-- looking for patient compliance in substitution therapy (EDDP, Norbupre.)

-- excluding additional drug consumption from patients in „clean-therapy“

-- drug testing in prisons

-- "workplace testing“, occupational medicine

-- psychiatric patients, therapeutic drug addiction


stopper(S)
swab(W)
(EHG)
(V)
put together
how it comes

Instructions

1. Take cotton wool swab from Salivette.
2. Chew the swab or put under the tongue (5min).
3. Put wet swab into the insert and close Salivette with stopper.
4. Put barcode label on the Salivette and send to lab

Lab

4. Weighing whole Salivette (eSLG)
5. Centrifuge at 5000 rpm for 10 min
6. Take insert with swab from Salivette
7. Weighing stopper+centrifuge vessel (R), calculate volumes
8. Use clear saliva for EIA and confirmation

Formula:

R [g]	= S + V
SLG [g]	= S + W + EHG + V
bSV[ml]	= eSLG - $\overline{SLG}$
nSV [ml]	= eR - $\overline{R}$
T [ml]	= bSV - nSV

SLG	= Salivettenleergewicht
eSLG	= eingespeicheltes Salivettenleergewicht
EHG	= Einhängegefäß
R	= Reservoir
eR	= eingespeicheltes Reservoir
S	= Stopfen
T	= Totvolumen
W	= Watterolle
V	= Vorlage
bSV	= Bruttospeichelvolumen
nSV	= Nettospeichelvolumen

Weighing data

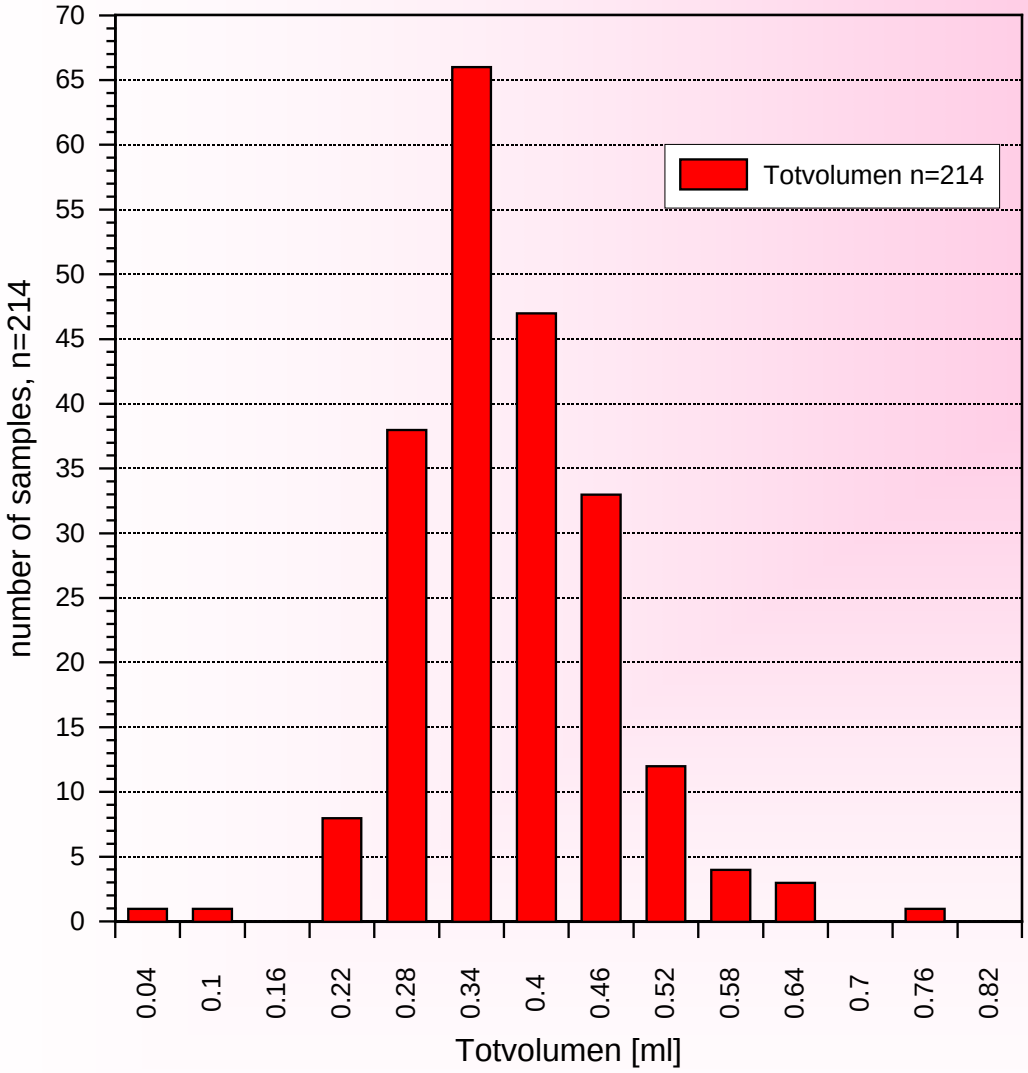
n = 150	**R**eservoir (S + V)	whole Salivette SLG
mean	4.57 g	6.72 g
median	4.56 g	6.72 g
SD	0.036	0.060
RSD	0.8%	0.9%

Cotton wool swab: dead volume

n = 20	**T**otvolumen (T)	**N**etto**S**aliva**V**olume (nSV)
mean	0.34 g	2.93 g
median	0.34 g	2.99 g
SD	0.05	0.206
RSD	15%	7%

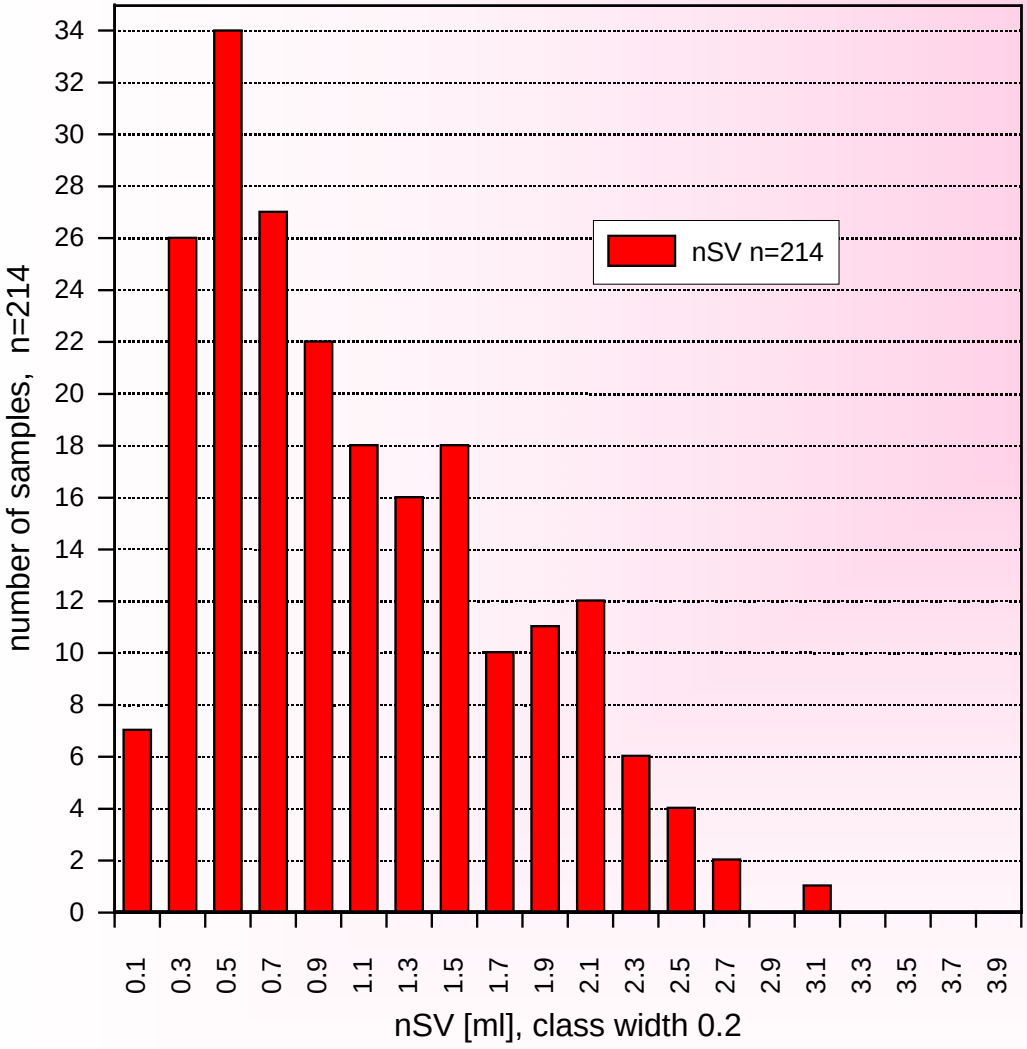
Swab rolled in saline for 5min

Salivette: dead volume



n = 214 26 individuals	Totvolumen (T)
mean	0.37 g
median	0.36 g
SD	0.079
RSD	21%

Salivette: NettoSalivaVolume



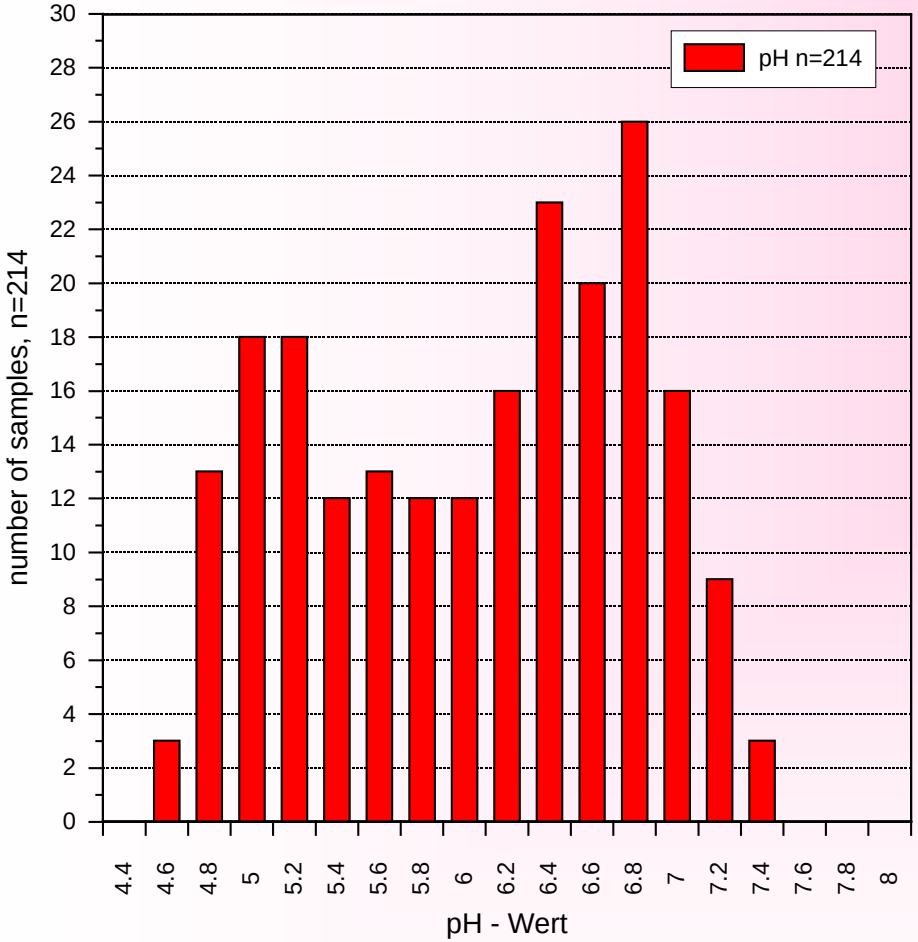
n = 214 26 individuals	NettoSalivaVolume (nSV)
mean	1.07 g
median	0.95 g
SD	0.644
RSD	**60%**

95% Perzentile: 2.3
5% Perzentile: 0.25

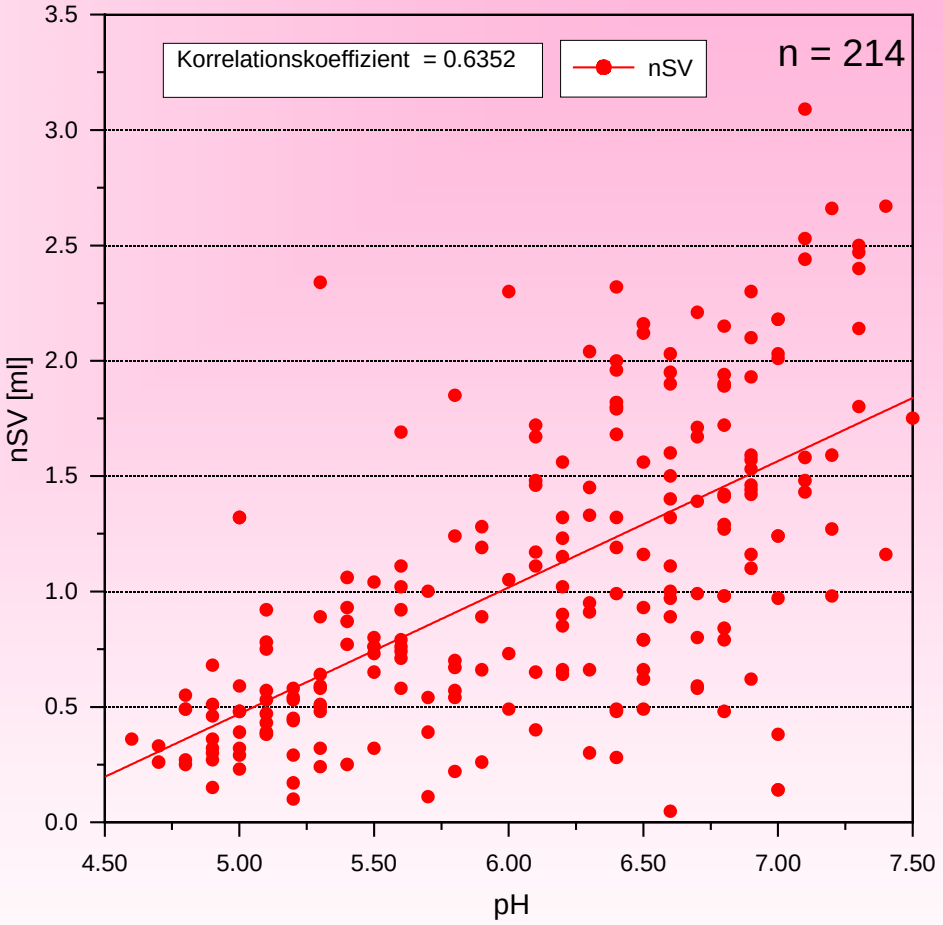
Routine:

if nSV = 0 mL (opiate addicts!) elute (bSV?) swap with 1mL 0.9%NaCl, look for Amylase activity, perform screening

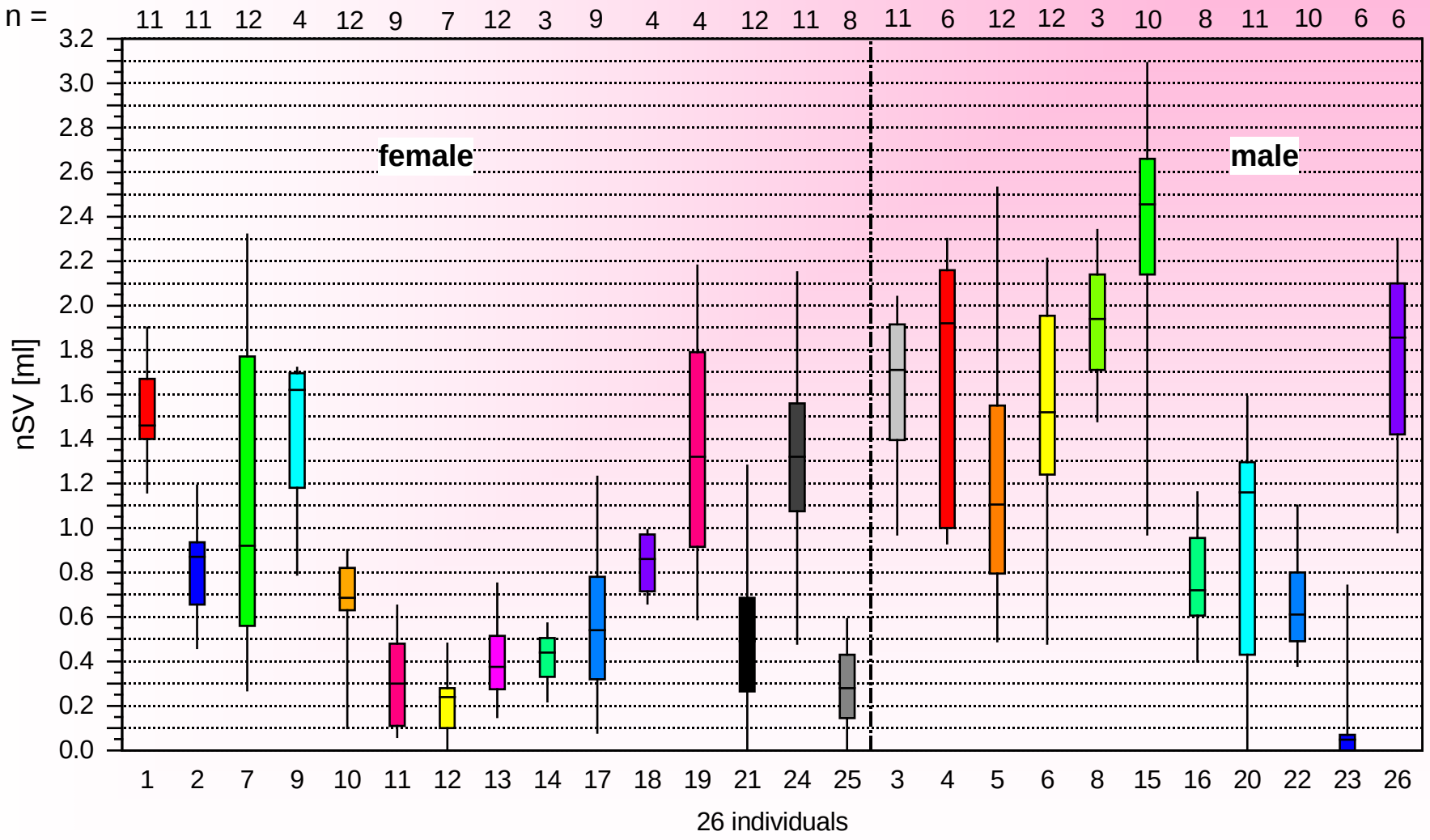
Variability of pH



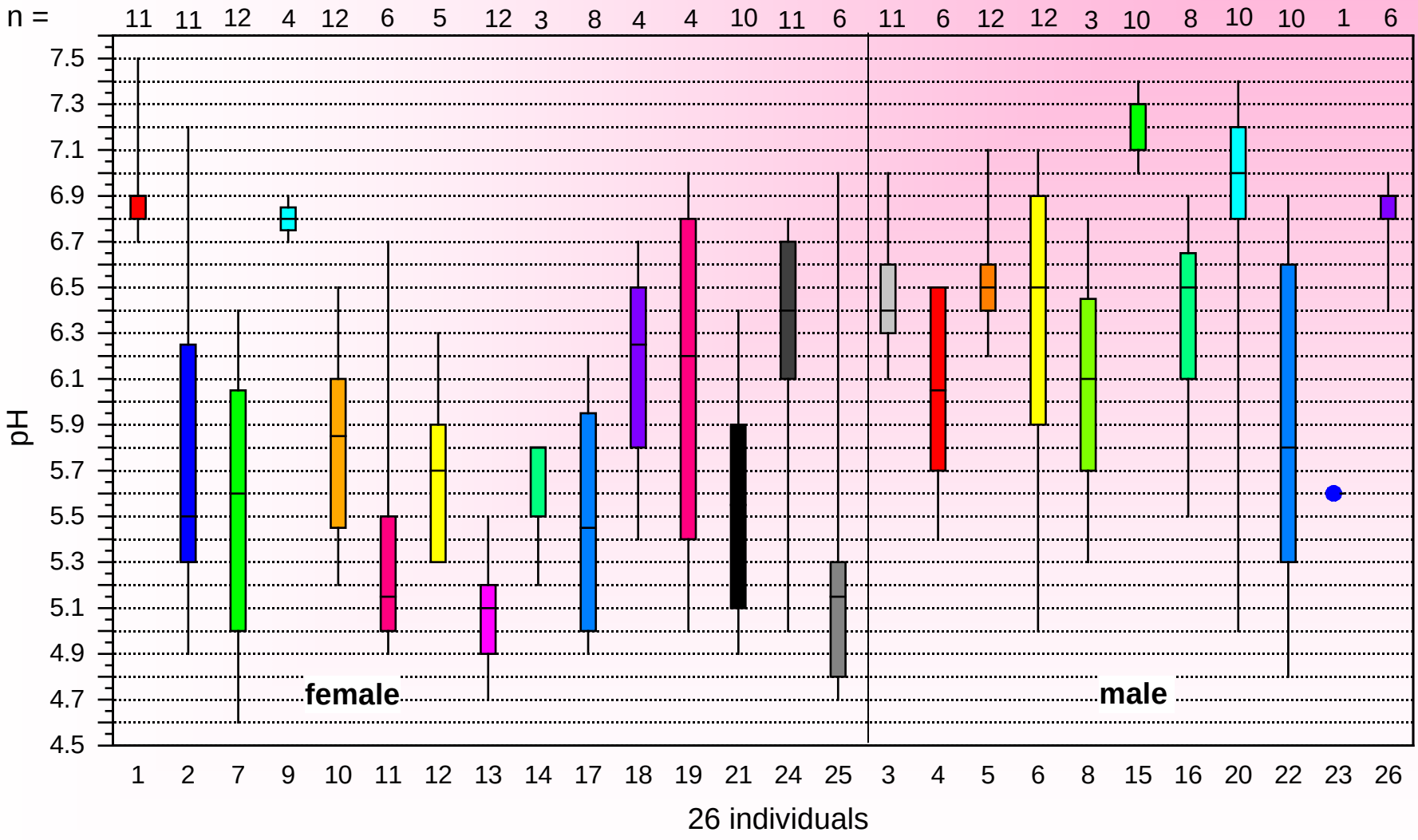
pH related to nSV (saliva flow)



Salivette
Intra- /inter-individual variability of nSV



Salivette
Intra- /inter-individual variability of pH



Salivette disadvantages

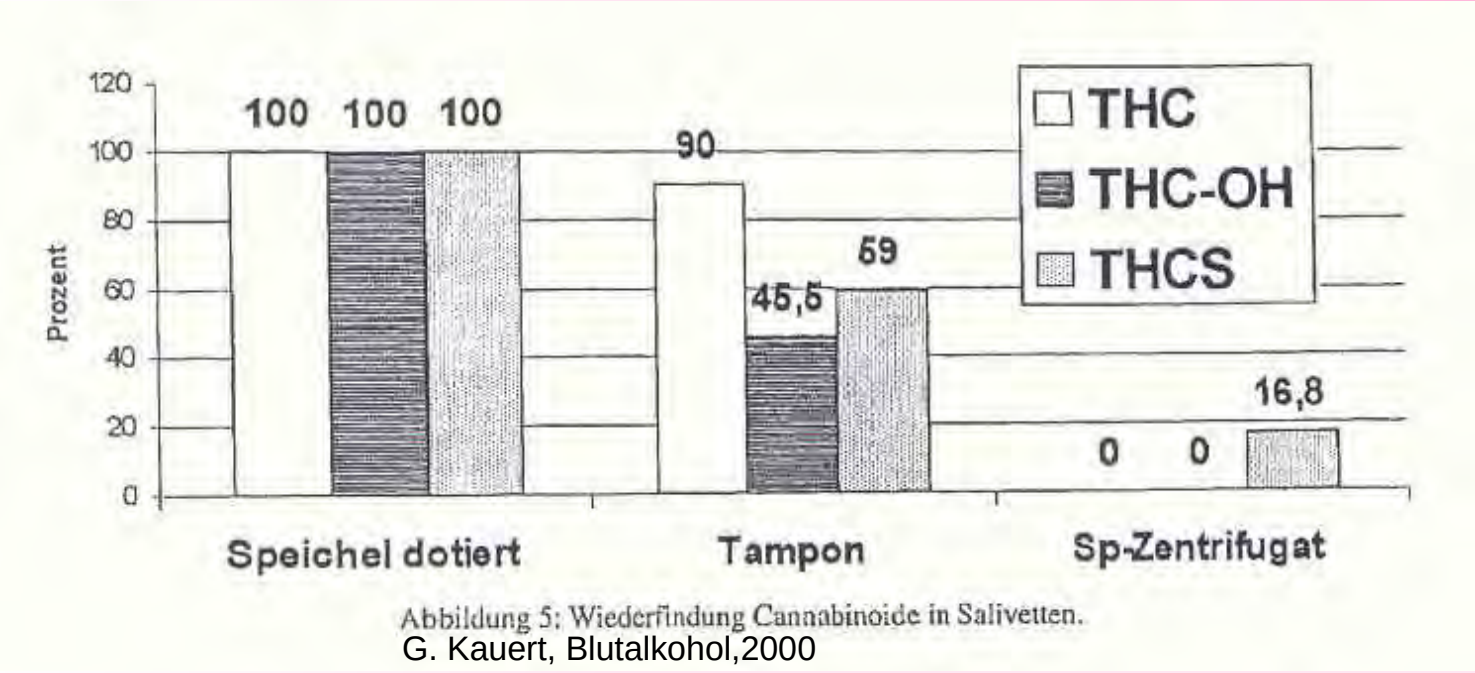


Abbildung 5: Wiederfindung Cannabinoide in Salivetten.

G. Kauert, Blutalkohol,2000

-- THC is adsorbed, elution with methanol possible
-- highly variable saliva volume
-- 21% of Salivettes were dry (Kauert), corresponds to our experience (Amylase!!)

Intercept (Orasure) Oral Fluid collection device

OMT =

Oral Mucosal Transudate
= „ultra-filtrate from the
that leaks from the crevic
and mucosal membranes
the mouth“

Instruction

1. Take pad from packaging.

2. Place collection pad between the lower cheek and gums. Gently rub the pad back and forth until pad is moist.

3. Once moist, leave collection pad between cheek and gums for a full 2 min.

4. Put pad into specimen vial and snap the wand at the scored line against the side of the vial (cave!).

5. Close specimen vial and send to lab.

Lab

5. Snap off tip from the specimen vial. Centrifuge at 2500 rpm for 15 min into 10mL vial (mean weight = 6.21 g).

6. ***Weigh vial and calculate OMT/buffer volumen and OMT volume.***

8. Use OMT/buffer for EIA and confirmation. ***EIA calibration better performed in buffer.***

Sample in buffer claimed to be stable for 3 weeks at RT

Letter to the Editor

Comment on Oral Fluid Testing for Drugs of Abuse: Positive Prevalence Rates by Intercept™ Immunoassay Screening and GC–MS–MS Confirmation and Suggested Cutoff Concentrations

To the Editor:

The recent paper "Oral Fluid Testing for Drug of Abuse: Positive Prevalence Rates by Intercept Immunoassay Screening and GC–MS–MS Confirmation and Suggested Cutoff Concentrations" (1) provokes some serious concerns for oral fluid drug testing.

1. Specifically, the device in question (Intercept) collects oral fluid, but apparently exactly how much is unknown. Therefore, it is not feasible to recommend cutoff concentrations based on this device because they would not be relevant or applicable to other collection devices and the units are not valid (ng/mL of oral fluid).

The authors say that the average volume collected is 400 µL, but the possible range of oral fluid volume is 50–800 µL. The potential for erroneous results, most likely false negatives based on insufficient sample collection, is obvious. This test is measuring drug in an unknown volume of oral fluid, which may or may not be approximately 0.4 mL. This paper provides a reference point for the Intercept device only, and so the cutoffs the authors suggest cannot be applied to other collection devices.

2. Laboratory guidelines generally allow for a ± 20% variation in measurements regarding analytical procedures (above and below cutoffs, ion ratios, etc.). The 50% variation in collection volume of this device is unacceptable by any standard procedure.

3. The authors argue that there is variability associated with urine collection due to individual differences, but this is also true for salivation. However, urine is not further diluted with some sort of buffer, whereas the Intercept device compounds the problem in individual salivation rates by introducing the buffer as yet another variable. How can the authors be sure that all the drug absorbed onto the sample pad is removed into the buffer?

4. The reference to measurement of IgG in the sample misleads the reader. The presence of adequate human IgG in the specimen means it is a valid sample for HIV testing, not that there is adequate volume of oral fluid for drug testing.

5. The original cutoffs for oral fluid first recommended to the SAMHSA working group by some of these authors, multiplied their result by 4 in order to set cutoffs, not by 3 as is the case in this paper. Has something changed in the Intercept device so as to change the original recommendations?

In order to validate the results presented in this publication, neat oral fluid collection and Intercept collection should be performed simultaneously. The results can then be compared and the device accepted as providing information in comparison to neat oral fluid.

Although we agree with the authors that oral fluid testing has distinct advantages over urinalysis, their rationale for suggesting cutoff concentrations is not valid because they have no idea how much oral fluid is actually being tested. To recommend nationwide cutoffs based on the device of one manufacturer is inappropriate, especially when the quantitative results obtained are highly questionable.

We would welcome further dialogue from the authors as well as others in the field.

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References

1. E.J. Cone, L. Presley, M. Lehrer, W. Seiter, M. Smith, K.W. Kardos, D. Fritch, S. Salamone, and R.S. Niedbala. Oral fluid testing for drugs of abuse: positive prevalence rates by Intercept™ immunoassay screening and GC–MS–MS confirmation and suggested cutoff concentrations. *J. Anal. Toxicol.* **26:** 541–546 (2002).

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Assay of Δ^9-Tetrahydrocannabinol (THC) in Oral Fluid—Evaluation of the OraSure Oral Specimen Collection Device

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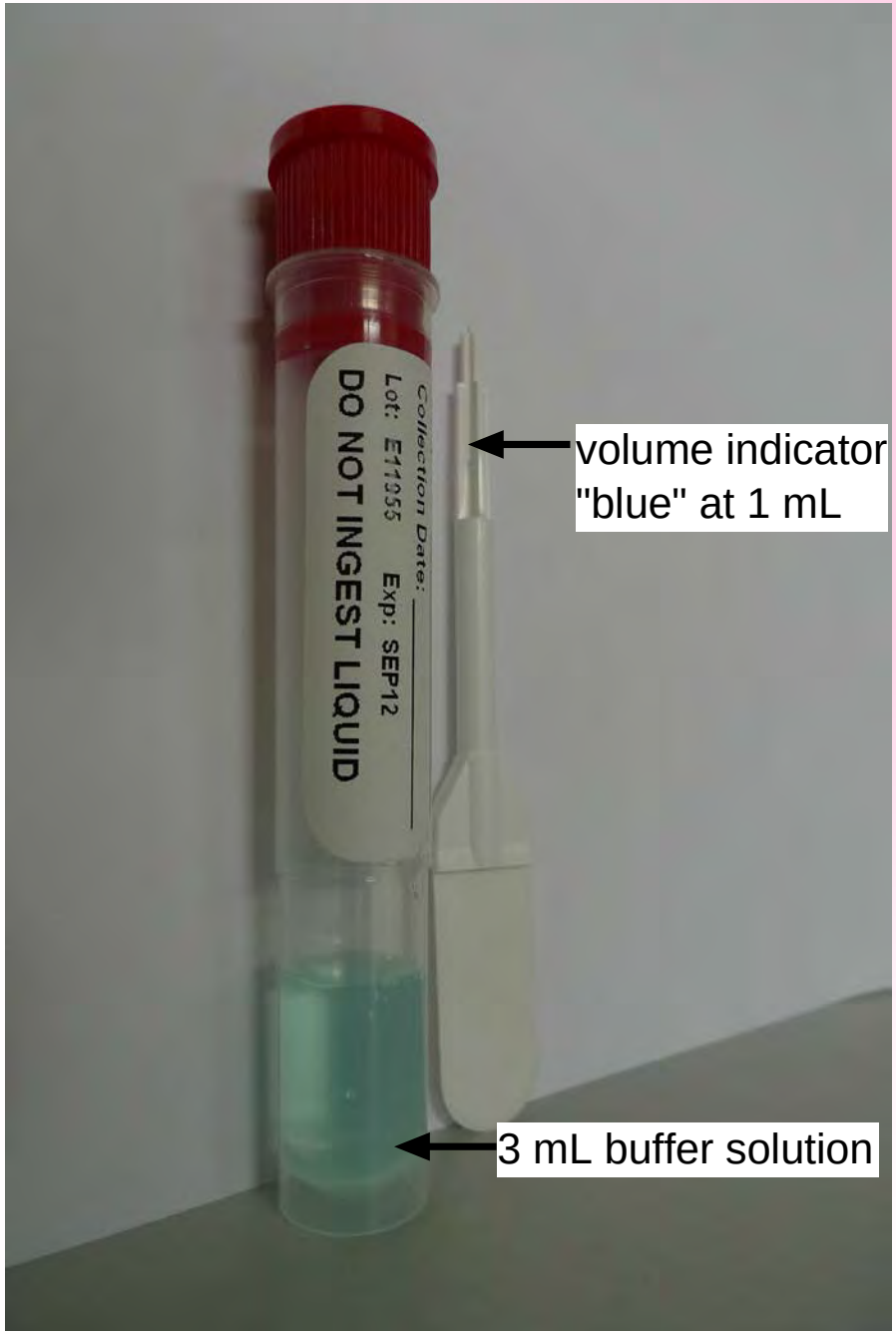
Abstract

Oral fluid is considered to be an alternative to urine testing for the detection of acute ingestion of drugs. The OraSure Intercept DOA Oral Specimen Collection Device (OSCD) has been used in studies for the quantitation of Δ^9-tetrahydrocannabinol (THC), but concerns have been raised. In the present study, we investigated whether the volume of oral fluid can be determined and how much THC remains adsorbed on the device. It was found that THC is markedly adsorbed onto the absorptive pad. The recovery using the standard elution procedure was only 37.8 ± 9.4% for 10 ng/mL and 55.6 ± 1.0% for 100 ng/mL of THC in oral fluid (n = 5 each). With an additional methanol wash, a further 25% could be eluted. Therefore, a modification of the procedure was evaluated, consisting of the addition of 2 mL of methanol to the elution buffer. THC could be completely recovered over a range of concentrations (1 to 1000 ng/mL). For the determination of the amount of oral fluid absorbed, a gravimetric approach was evaluated as the weights of the devices vary only by 0.6% relative standard deviation. After application of 0.5 mL oral fluid to pads and evaluation of the weight differences, the applied amount could be estimated with a precision of 7.5% (n = 8) and an accuracy of 6.1%. From these results it can be concluded that the OraSure OSCD is useful to collect oral fluid for reliable quantitative THC assay applying a modified elution procedure and gravimetric determination of the amount of oral fluid.

Quantisal QS



contains detergent

Quantisal™

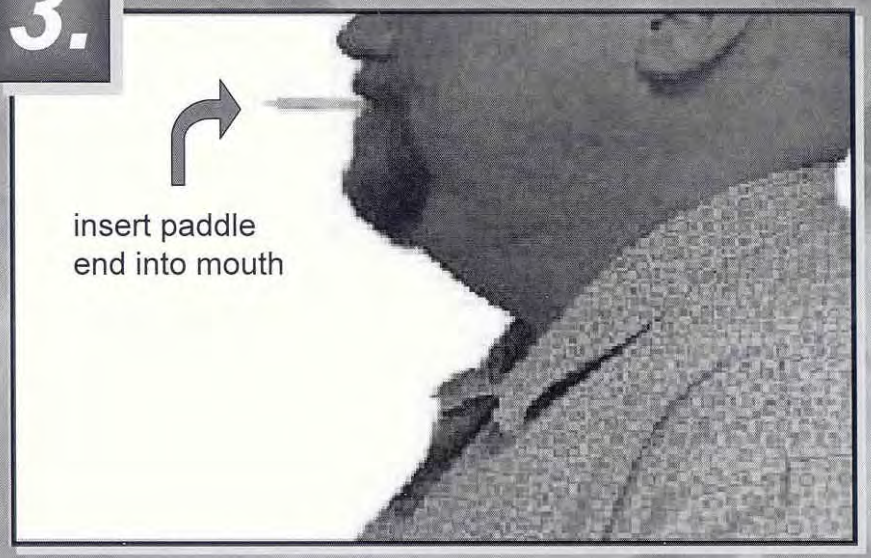
- Provides rapid, reliable collection of a FULL one milliliter of saliva using its *Volume Adequacy Indicator*
- No stimulants on the collection pad to cause the donor to salivate: this means that it meets SAMSHA "no saliva stimulation" regulation and has no "bad taste"

IMMUNALYSIS

+-10%

Quantisal™ - Collection Procedure

3.



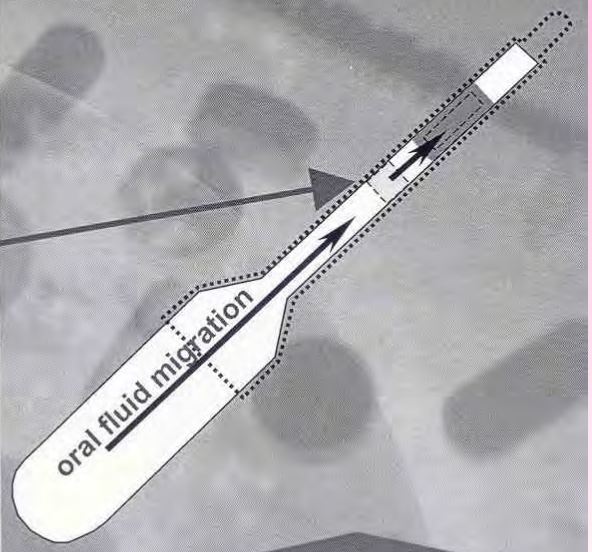
Instruct donor to position collection paddle under tongue and close mouth.

IMMUNALYSIS

Quantisal™

Volume Adequacy Indicator - How Does it Work?

- As the cellulose pad saturates with oral fluid, the fluid migrates along the cellulose tail and dissolves the dye.
- As the pad further saturates, the dye migrates further up the tail until it becomes visible in the window of the stem.



IMMUNALYSIS

Quantisal™ - Collection Procedure

Issues with Donors that Use Tobacco

- Smokers and other tobacco users should rinse their mouths *before* inserting the Quantisal™. These donors have "drier mouths" or thicker oral fluid than non-smokers/non-tobacco users.

- In collections from smokers and other tobacco users, the "volume adequacy" indicator may take longer to turn blue (up to 10 minutes).

IMMUNALYSIS

Other drugs?

Quantisal™ - Donor Instructions

1. Donor is not to talk while Quantisal™ is in his/her mouth. The jaw movement can damage the pad.
2. Donor is NOT to bite on the pad. This can shred the pad.
3. Donor is NOT to bite on the stem. This can block the oral fluid from reaching the blue "volume adequacy" indicator.

IMMUNALYSIS

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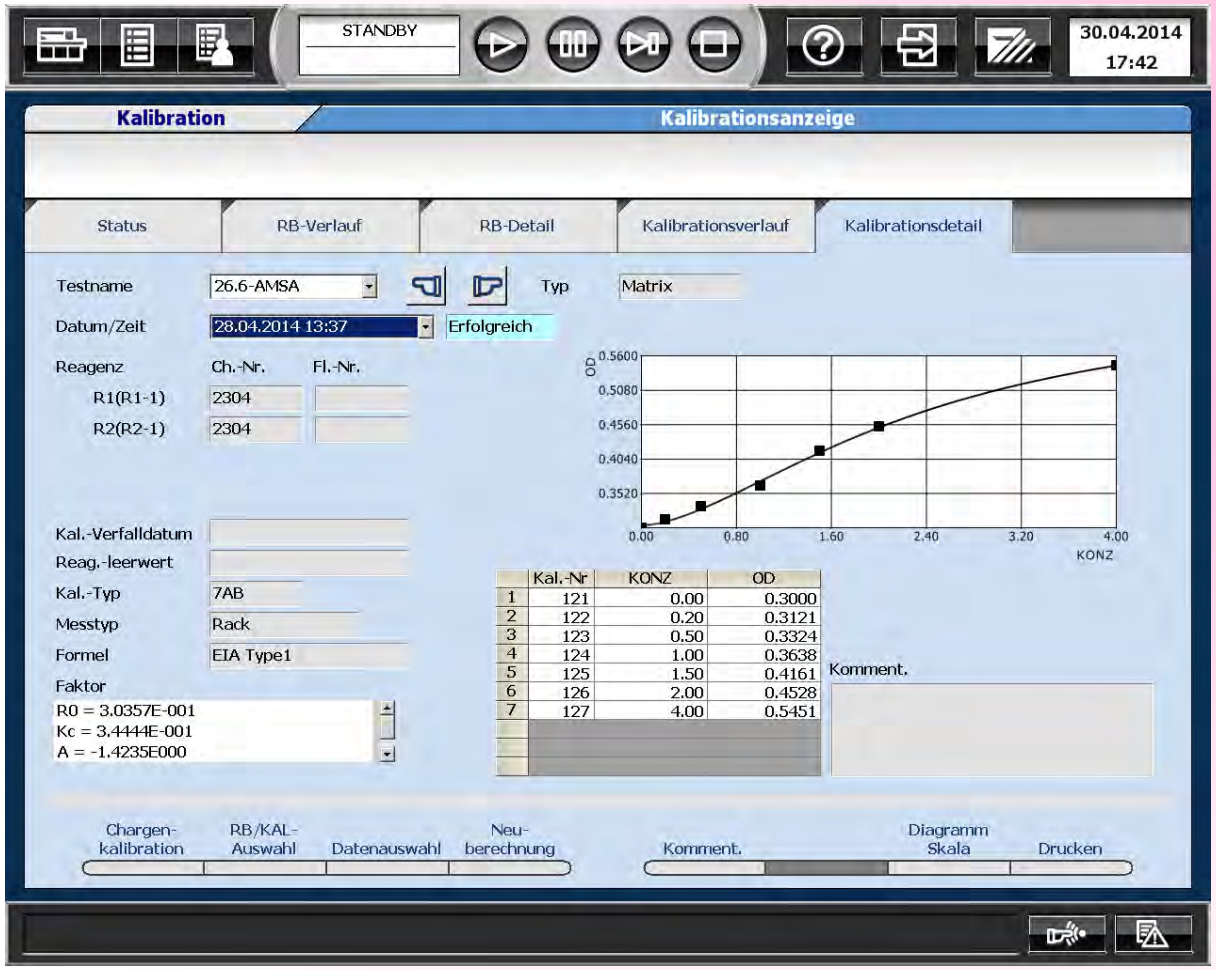
Calibration 6-Acetylmorphine in SA/SES 50%



Traktorist: MT

Comments:

Calibrator 1 ; 0 ng/mL

MGC Negative Calibrator

Order no.: 1557416

Reference method : **0 ng/mL**

LOT:

Expiry date:

Lot in use:

Bottle in use:

Calibrator 2 ; 0.2 ng/mL

Order no.:

Reference method : **0.2 ng/mL**

LOT:

Expiry date:

Lot in use:

Dilution date:

Calibrator 3 ; 0.5 ng/mL

Order no.:

Reference method : **0.5 ng/mL**

LOT:

Expiry date:

Lot in use:

Dilution date:

Calibrator 4 ; 1.0 ng/mL

Order no.:

Reference method : **1.0 ng /mL**

LOT:

Expiry date:

Lot in use:

Bottle in use:

Calibrator 5 ; 1.5 ng/mL

Order no.:

Reference method : **1.5 ng /ml**

LOT:

Expiry date:

Lot in use:

Dilution date:

Calibrator 6 ; 2.0 ng/mL

Order no.:

Reference method : **2.0 ng/mL**

LOT:

Expiry date:

Lot in use:

Dilution date:

Calibrator 7 ; 4.0 ng/mL

Order no.:

Reference method : **4.0 ng/mL**

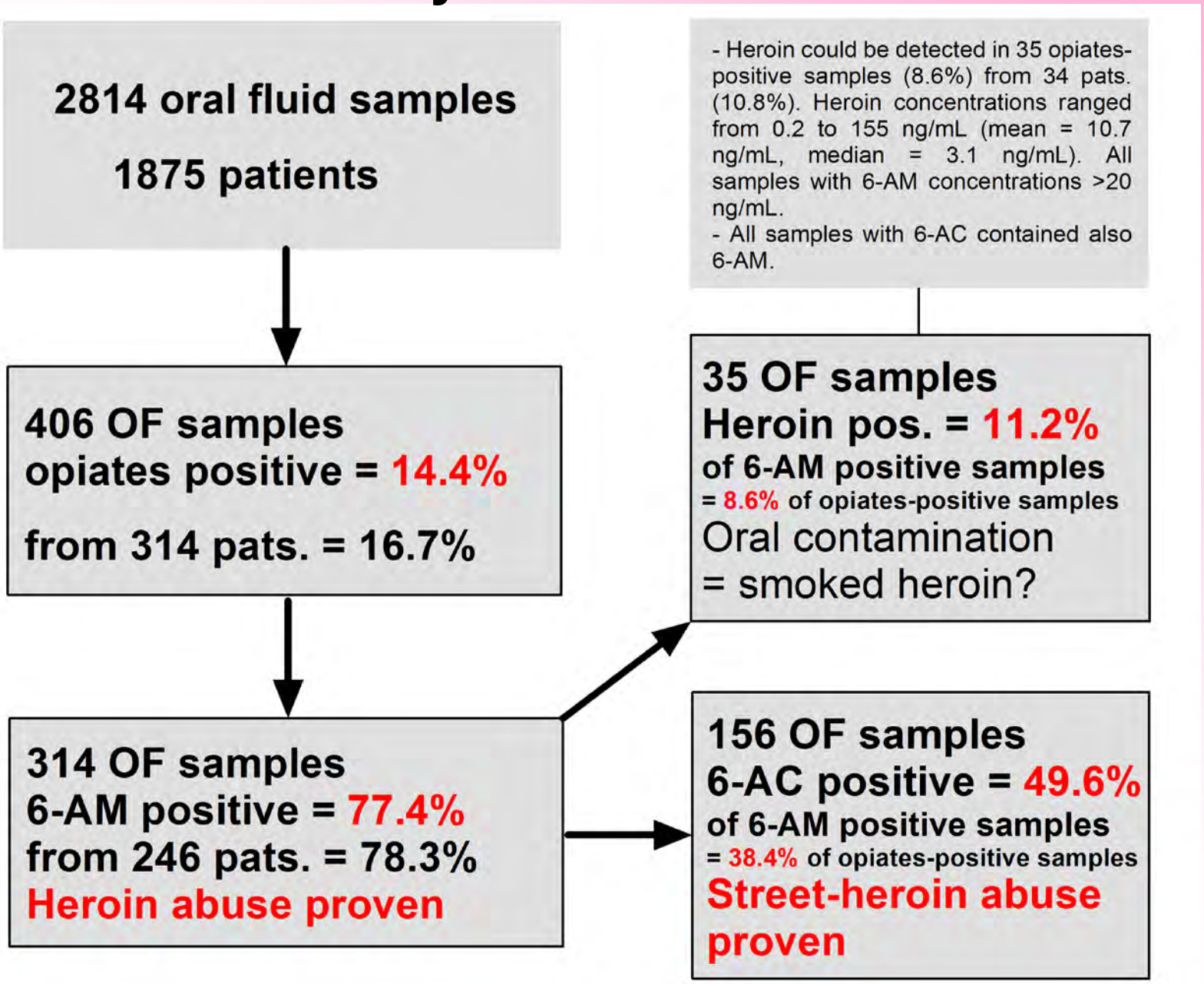
LOT:

Expiry date:

Lot in use:

Dilution date:

3rd study: smoked heroin?



Substitution drugs:

	Cutoff 0.1 ng/mL	Cutoff 1 ng/mL	Cutoff 10 ng/mL
EDDP	3671 (68.5%)	3031 (56.6%)	698 (13.0%)
pos. rate reduced		**17.4%**	**81.0%**
Methadone		3671 (68.5%)	3660 (68.3%)
pos. rate reduced by			**0.3%**
Norbuprenorphine	1283 (24.0%)	822 (15.4%)	44 (0.8%)
pos. rate reduced by		**35.9%**	**96.6%**
Buprenorphine		1283 (24.0%)	615 (11.5%)
pos. rate reduced by			**52.0%**

In compliance testing unintentional oral contamination (nurse: sampling post dosing) must be differentiated from intentional oral contamination by the patient ("self" dosing prior sampling). Therefore the concentration of substitutes metabolites EDDP and Norbuprenorphine resp. should be "somehow" in agreement to the parent drug concentration. This esp. is of importance at high parent drug concentrations.On the other hand a false negative result for the metabolites could lead to falsely assumed non-compliance of the patient and must be avoided. This is of importance when regarding pats. in low-dose therapy. At the 0.1 ng/mL CO EDDP and Norbuprenorphine will be detected when the patient is in steady-state.

next matrix:
drugs in exhaled breath.....?